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Effects of Carbon Monoxide on Myocardial Ischemia

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The purpose of this study was to determine whether low doses of carbon monoxide (CO) exacerbate myocardial ischemia during a progressive exercise test. The effect of CO exposure was evaluated using the objective measure of time to development of electrocardiographic changes indicative of ischemia and the subjective measure of time to onset of angina.

Sixty-three male subjects (41–75 years) with well-documented coronary artery disease, who had exertional angina pectoris and ischemic ST-segment changes in their electrocardiograms, were studied. Results from three randomized, double-blind test visits (room air, low and high CO) were compared. The effect of CO exposure was determined from the percent difference in the end points obtained on exercise tests performed before and after a 1-hr exposure to room air or CO. The exposures resulted in postexercise carboxyhemoglobin (COHb) levels of 0.6% ± 0.3%, 2.0% ± 0.1%, and 3.9% ± 0.1%. The results obtained on the 2%-COHb day and 3.9%-COHb day were compared to those on the room air day. There were 5.1% ($p = 0.01$) and 12.1% ($p \leq 0.0001$) decreases in the time to development of ischemic ST-segment changes after exposures producing 2.0 and 3.9% COHb, respectively, compared to the control day. In addition, there were 4.2% ($p = 0.027$) and 7.1% ($p = 0.002$) decreases in time to the onset of angina after exposures producing 2.0 and 3.9% COHb, respectively, compared to the control day. A significant dose-response relationship was found for the individual differences in the time to ST end point and angina for the pre- versus postexposure exercise tests at the three carboxyhemoglobin levels. These findings demonstrate that low doses of CO produce significant effects on cardiac function during exercise in subjects with coronary artery disease.

Introduction

Regulatory Background of the Study

A study of the effect of carbon monoxide (CO) exposure on subjects with stable angina pectoris was undertaken at three clinical centers to determine whether or not relatively low doses of CO would result in myocardial ischemia. The purposes were to test the hypothesis that low doses of CO can affect cardiac function in individuals with documented coronary artery disease and to provide objective electrocardiographic (ECG) information for consideration in setting the National Ambient Air Quality Standards (NAAQS) for CO.

The NAAQS for CO, promulgated by the U.S. Environmental Protection Agency (EPA) in 1971, are set at levels of 9 ppm for an 8-hr averaging time and 35 ppm for a 1-hr averaging time. Extrapolation of these exposure levels to carboxyhemoglobin (COHb) levels is useful because COHb provides a biological marker that represents the best measurement of CO dose in individuals and enables the comparison of results among studies that employed different exposure regimens. According to estimates by the EPA (*1*), a typical adult involved in moderate activity would have a COHb level of approximately 2.0% after a 1-hr exposure to CO at

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35 ppm. Results from exposure models estimate that 60% of individuals with cardiovascular disease would exceed these levels of COHb (*2*). In the ambient atmosphere, CO levels usually fluctuate over the 8-hr averaging time. Various patterns of CO exposure that result in a 9-ppm 8-hr average would produce COHb concentrations in the range of 1.4 to 1.9% in typical adults.

Originally, the CO standards were based on human neurobehavioral studies by Beard and Wertheim (*3*). These investigators reported impairment in the ability to discriminate time intervals at COHb levels as low as 1.8%. Although later studies did not support alterations in vigilance at levels below about 5% COHb (*4*), other data suggested effects in humans at COHb levels of 2 to 3%. Subsequent studies by Aronow and Isbell (*5*) and by Anderson and co-workers (*6*), which showed a decrease in the time to the onset of angina (chest pain) during exercise in subjects with coronary artery disease, were used to justify the NAAQS for CO.

Aronow and Isbell (*5*) reported a statistically significant reduction in time to angina in a group of 10 subjects with coronary artery disease who had average COHb concentrations of 2.7% after a 2-hr exposure to 50 ppm CO. The study design involved comparing the time to onset of angina during exercise tests administered before and after exposure either to clean air or to air containing elevated levels of CO. In a later study, Aronow (*7*) reported a similar decrease in the time to angina during exercise in 15 subjects after exposure to 50 ppm CO for 1 hr, resulting in average COHb levels of only about 2.0%. Other evidence for an effect of CO at COHb levels less than 3% was provided by a study of 10 subjects with angina by Anderson and co-workers (*6*). The Anderson study had a somewhat different design than the Aronow studies (*5,7*) in that there was no pre-exposure exercise test. On different days, each subject was exposed to normal air, air with 50 ppm CO, or air with 100 ppm CO for 4 hr and had average postexposure blood COHb levels of 1.3, 2.9, and 4.5%, respectively. Average exercise time was significantly shortened by the same amount after either CO exposure.

Given the small number of subjects and the subjective nature of the end point (angina pectoris) in the Aronow and Anderson studies (*5–7*), further investigation of the health effects of CO was indicated. In addition, an expert committee convened by the EPA concluded that the EPA should not consider the Aronow results as definitive evidence of the biologic effects of CO, but might consider using them in developing a margin of safety (*1*). Because of the potential importance of reports showing CO effects on subjects with angina pectoris in the justification of the NAAQS for CO, a study was organized by the Health Effects Institute (HEI) to provide objective measurements of the potential effect of CO exposure in subjects with coronary artery disease (*8,9*). Specifically, the study was designed to answer the question: Do CO exposures that produce 2 or 4% COHb decrease the time to onset of ischemia during exercise, as documented by ECG ST-segment changes and the onset of angina?

Scientific Basis of the Study

Carbon monoxide's toxicity is related to its ability to bind tightly to hemoglobin, forming COHb. This reaction not only decreases the oxygen-carrying capacity of the blood, but also shifts the oxyhemoglobin (O_2Hb) dissociation curve to the left (*10*). The shift to the left means that oxygen is released less readily from circulating hemoglobin, thereby reducing oxygen delivery to peripheral tissues. The net consequence of these effects is to produce a state of relative hypoxia in the tissues. Other mechanisms of toxicity may also exist. Use of COHb as the measure of dose does not imply that a decrease in the oxygen-carrying capacity of the blood is the sole mechanism for effects of CO.

Low doses of CO (< 5% COHb) are generally not associated with adverse effects in normal individuals. However, there are several conditions that may make an individual more susceptible to the adverse effects of CO exposure. These include exposure to high altitude, where ambient oxygen tension is reduced; anemia, where the oxygen-carrying capacity of the blood is decreased; chronic lung disease, where gas-exchange abnormalities cause hypoxemia; and occlusive vascular disease, in which blood flow to the tissue is restricted. The last condition, as it pertains to the heart, is the focus of this study.

The hypothesis tested in this study is that low doses of CO reduce exercise performance in subjects with coronary artery disease. The rationale for this hypothesis is based upon the inability of an individual with coronary artery disease to increase coronary blood flow sufficiently to meet increased levels of myocardial oxygen consumption. Increased myocardial oxygen demand is normally met primarily by increasing coronary blood flow because the extraction of oxygen by cardiac muscle, even at rest, is near maximum. As a subject exercises, myocardial oxygen demand increases in order to maintain an adequate cardiac output. As long as the increasing myocardial oxygen demands are met, the heart continues to function normally. At the point where myocardial blood flow cannot meet oxygen demands, the myocardium becomes ischemic. Altered cellular metabolism associated with myocardial ischemia results in the development of chest pain or characteristic ECG changes, or both. Symptoms of myocardial ischemia (angina pectoris) occur in individuals with coronary artery disease at specific levels of exercise and progress to levels that limit their exercise capacity. It is possible that increased levels of COHb may produce ischemic symptoms at lower levels of work because of the reduced oxygen-carrying capacity of the blood.

Methods

Study Design

The goal of this study was to evaluate the effect of low doses of CO, producing 2 and 4% COHb, on subjects

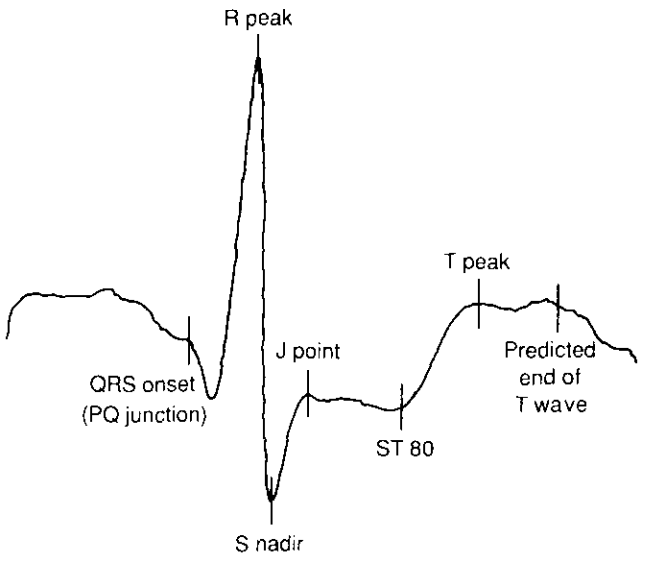

FIGURE 1. Trace of a normal ECG pattern displaying ST segment.

Table 1. Protocol for qualifying and test visits.

Qualifying visit (visit 1)	Test visits (visits 2, 3, 4)
History, physical exam	History, physical exam
COHb level	COHb level
Exercise test 1	Exercise test 1
Sham exposure	Randomized exposure
	Air
	CO, 2% COHb
	CO, 4% COHb
Exercise test 2	Exercise test 2
CO uptake exposure	

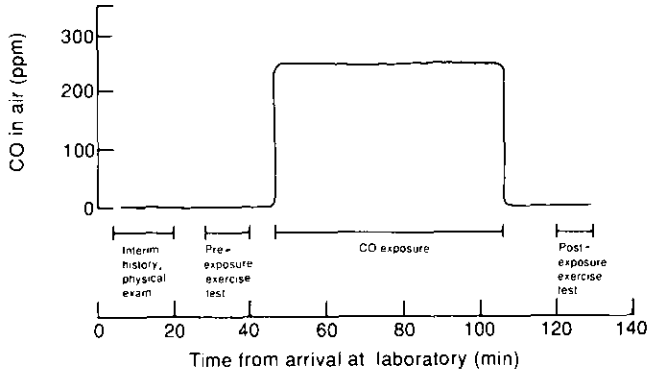

FIGURE 2. Illustration of protocol for test visit on 4%-COHb target day.

with coronary artery disease. Two end points were used to assess adverse effects. The time to development of an ST-segment change in the ECG (Fig. 1) that exceeded a specific threshold level (see "Exercise Treadmill Testing" under "Methods") will be referred to as time to "ST end point." The ST end point was chosen because it is an objective indicator of myocardial ischemia. The second end point was the time to onset of angina pectoris. This provides an indicator of symptomatic myocardial ischemia and was the primary cardiac end point used in earlier studies.

Subjects enrolled in this study had coronary artery disease with stable exertional angina and reproducible exercise-induced ST-segment changes. To ensure an adequate sample size, 20 to 25 subjects were studied at each of three centers. A standardized protocol was developed, and the same experimental methods were followed at each center.

The study consisted of four visits for each subject: a base-line qualifying visit (visit 1) and three randomized, double-blind test visits (visits 2, 3, and 4). The protocol for the qualifying and test visits is outlined in Table 1. Figure 2 illustrates the protocol for a test visit. The basic design of the study was to use repeated exercise tests each day to minimize variability in end points (*11*). On each test day, the subject was screened for elevated base-line COHb levels, performed a symptom-limited exercise test on a treadmill, after recovery was exposed in an environmentally controlled chamber for approximately 1 hr to room air or CO in room air, and then performed a second exercise test. The exposure conditions for test visits were randomized among room air and two levels of CO expected to produce 2.2 and 4.4% COHb before exercise and approximately 2.0 and 4.0% at the end of exercise. Based upon pilot experiments in normal subjects, a 10% decrease in percent carboxyhemoglobin during exercise was projected.

The analysis was designed to provide maximal power to reduce the number of subjects required to test the hypothesis that low doses of CO reduce exercise performance. For each test day, the difference in results of the pre- and postexercise tests for the two end points (time to ST end point and time to angina) was determined, and then these differences on the 2- and 4%-COHb-target days were compared to the results on the control day. This analysis is preferable to a direct comparison of the time to end point on different test days because of greater day-to-day variation than within-day variation of these two end points in these subjects (*11*). The protocol was reviewed by the Institutional Review Board at each test center. Throughout the methods section, values are presented as means ± standard deviations.

Visit 1. The qualifying visit (Table 1) included obtaining the subject's informed consent, followed by a complete medical history and physical examination and a baseline 12-lead ECG. If the subject qualified by the inclusion-exclusion criteria described below, a blood sample was taken to determine whether or not the subject's baseline blood COHb level was above the level specified by the exclusion criterion. This requirement is identical to that in visits 2, 3, and 4, and is dis-

cussed in detail below. A symptom-limited exercise treadmill test, using a modified Naughton protocol (*12*) was then performed. After the exercise test, the subject rested until his ECG returned to baseline values. The subject then entered the environmental chamber for a 1-hr sham exposure period prior to a second, identical exercise test. Qualification for entry into the study consisted of reproducible results on the two exercise tests: Both tests had to be positive with respect to both ST end point and angina; in addition, the difference in duration of the two exercise tests had to be 150 seconds or less.

Subjects who met the entrance criteria were then exposed in the environmental chamber to 150 ppm CO for 60 min with full knowledge of the exposure conditions. The subjects remained seated during the exposure. Venous blood samples were collected every 15 min and analyzed immediately for %COHb by CO oximetry. These data were used to determine the subject's CO uptake constant; this was used on subsequent visits to calculate the atmospheric CO levels required for each subject to absorb sufficient CO in 1 hr to reach the desired COHb level.

Visits 2, 3, and 4. Subjects who met the reproducibility requirements during the initial visit returned for three test visits (Table 1). These visits were separated by at least 72 hr, and all occurred within a 4-week period. The subjects were instructed to continue their cardiac medications throughout the study. Every effort was made to maintain consistency of dose and time of use before a test visit. Subjects were also instructed to refrain from eating or drinking for 2 hr before coming to the laboratory.

Upon arrival at the laboratory, the subject's baseline COHb level was measured by CO oximetry to assure that it was at or below the equivalent of the gas chromatography (GC) level of 1% (see below). A pretest interim history and 12-lead ECG were done at each visit to verify that the subject was clinically stable. The subject then performed a symptom-limited exercise test and was allowed to recover. The original protocol permitted a subject to complete the test visit whether or not he achieved the ST end point or developed angina during the first exercise test. As a precaution, the protocol was amended, effective February 18, 1986, to minimize the potential failure to achieve the cardiac end points. When a subject did not develop angina or achieve the ST end point on the pre-exposure exercise test of visits 2, 3, or 4, the exposure and second exercise test for that day were not done, and the visit was rescheduled. If the subject failed to achieve one or both of the end points on two successive visits, he was dropped from the study. This resulted in rescheduling two subjects and dropping one subject from the study.

The subjects were exposed according to a randomization schedule (Appendix A) to room air, to the lower level of CO, or to the higher level of CO after recovering from the first exercise test. The subjects and the personnel responsible for the exercise testing and the cardiovascular monitoring were blinded as to the exposure conditions. Exposure personnel were not blinded but did not communicate with subjects or cardiology personnel. The exposure conditions were selected to produce no change in %COHb, an increase to 2.2% COHb (2%-COHb-target day), or an increase to 4.4% COHb (4%-COHb-target day), as measured by GC. These COHb targets are 10% greater than the desired levels at the end of the second exercise test, which were 2.0 and 4.0% COHb, to compensate for the loss of CO during exercise. In order to attain a relatively constant level of %COHb at the end of exposure, the level of CO in the chamber for each individual was varied according to the uptake constant determined during the qualifying visit (visit 1). An additional variable, duration of exposure, was also used to help attain the desired level of %COHb. All procedures used on the CO-exposure days were followed on the air-exposure days to maintain double-blind conditions. After the exposure, the subjects exercised using the same protocol for a second time. The mean time from the end of the exposure period to the beginning of the second exercise test was 17 min (SD = ± 10.2; SEM = ± 0.6).

Venous blood samples were collected to monitor the CO exposure conditions. Carboxyhemoglobin levels were measured by CO oximetry in each subject during visits 2, 3, and 4 at the times indicated in Table 2 and illustrated in Figure 3: arrival at the laboratory (sample 1); within 1 min of completing the first exercise test (sample 2); after 30 min of exposure (sample 3); after 40 min of exposure (sample 4); at the end of exposure (sample 5); and within 1 min of completing the second exercise test (sample 6). The protocol called for analysis of the data using GC based COHb levels. Therefore, key samples from all subjects were sent to the St. Louis Ref-

Table 2. Blood-sample collection and analysis for visits 2, 3, and 4.

Sample number	Collection time	Analysis	
		CO oximetry	Gas chromatography (at reference lab)
1	When subject arrives at lab	Immediately	No
2	At end of exercise test 1 (within 1 min)	Immediately	Yes
3	At 30 min of CO exposure	Immediately	No
4	At 40 min of CO exposure	Immediately	No
5	At end of CO exposure	Within 1 hr	Yes[a]
6	At end of exercise test 2 (within 1 min)	Within 1 hr	Yes

[a] The original protocol did not require GC analysis of sample 5. An amendment, with an effective date of August 29, 1985, added GC analysis of sample 5. Prior to that date, seven subjects were studied.

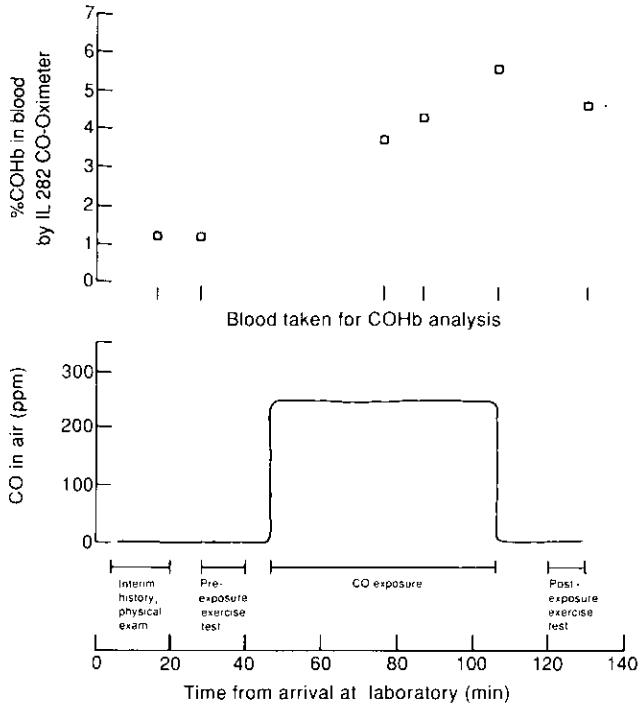

FIGURE 3. Sampling of blood during test visit. COHb levels in all samples were determined by CO oximetry. In addition, samples 2, 5, and 6 were analyzed for CO content by GC methods at the reference laboratory.

erence Laboratory for analysis by GC. Samples 2 and 6, which were drawn at the end of exercise tests 1 and 2, respectively, were analyzed by GC in all subjects throughout the study. Beginning August 29, 1985, a protocol amendment was introduced to require analysis by GC of sample 5 (end of exposure). Only CO oximetry measurements were performed on samples 1, 3, and 4.

To assure that the subjects did not encounter high levels of CO prior to arriving at the laboratory, sample 1 was collected and immediately analyzed. The initial upper limit for sample 1 was 1.8% COHb by CO oximetry, which was assumed to represent approximately 1% COHb by GC. As data accumulated during enrollment of the first subjects, comparison of COHb levels obtained by GC and CO oximetry resulted in protocol amendments that raised the upper limit allowable for sample 1. Fifty-six of the 63 subjects were tested using exclusion levels of COHb, measured by CO oximetry, of $> 2.0\%$ with O_2Hb levels of 60% or less, $> 2.2\%$ with O_2Hb levels of 61 to 80%, and $> 2.4\%$ with O_2Hb levels above 80%.

Samples 3 and 4 were used to project the subject's COHb level at the end of exposure at visits 2, 3, and 4. Each subject was exposed to a level of CO that should have resulted in the desired COHb level after 60 min, based on his uptake constant as measured at visit 1. If CO uptake measured at 30 and 40 min was faster than expected, then the exposure period was shortened accordingly. If uptake was slower, the exposure period was lengthened. Exposures were restricted to either 50, 55, 60, 65, or 70 min in length to limit the options for randomization on the air day.

Table 3. Major participants in the study.

Test Centers
Francis Scott Key Medical Center, The Johns Hopkins University School of Medicine, Eugene R. Bleecker, Sidney O. Gottlieb, Sandra M. Walden
Rancho Los Amigos Medical Center, Jack D. Hackney, Ronald H. Selvester, Robert B. Pearson
St. Louis University School of Medicine, Thomas E. Dahms, Bernard R. Chaitman, Robert Wiens
Reference Laboratory
St. Louis University School of Medicine, Thomas E. Dahms
Statistical and Data Management Center
Harvard University School of Public Health, Marcello Pagano, Elizabeth N. Allred
Quality-Assurance Group
Arthur D. Little, Inc., Denise Hayes, Andrew Sivak
Advisory Committee
John W. Tukey (Chairman), Stephen Achuff, Stephen Ayres, Joseph D. Brain, Steven Horvath, Roger O. McClellan
Project Manager
Health Effects Institute, Jane Warren

Organization of the Study

Many groups participated in this study, as shown in Table 3. Their roles are described briefly below.

Test Centers. Investigators at three medical centers (Johns Hopkins University School of Medicine, Rancho Los Amigos Medical Center, and St. Louis University School of Medicine) participated in designing the study, writing the protocol and standard operating procedures, and recruiting and testing the subjects.

Reference Laboratory. A reference laboratory, operated at St. Louis University School of Medicine, provided samples for comparing CO oximeter readings at the three test centers. The laboratory was also responsible for gas chromatographic measurements of CO on all key blood samples from each subject at the three test centers.

Statistical and Data Management Center. Investigators at the Statistical and Data Management Center at the Harvard School of Public Health participated in designing the study, writing the protocol, designing the data collection forms, and planning the data analysis. They also processed and analyzed data sent on forms from the test centers.

Quality Assurance Group. A group at Arthur D. Little, Inc., Cambridge, MA, was responsible for ensuring the quality of data generated in this study. This group participated in reviewing the protocol and standard operating procedures for the study. The Quality Assurance (QA) Officer performed site visits at the test centers, reference laboratory, and Statistical and Data Management Center in order to determine that the

protocol and standard operating procedures were being followed and that data were reliable and traceable. The QA group conducted the final quality assurance audit on the data at the end of the study.

Description of Subjects

Subject Selection Criteria. The selection criteria for the study population were chosen with the goal of obtaining a group of men who do not smoke and who have objective evidence of coronary artery disease, stable angina, and evidence of reproducible, exercise-induced myocardial ischemia (angina and ischemic ST changes on the ECG) during exercise treadmill testing. Women were excluded to reduce the risk of false-positive exercise test results that are more common in women (*13,14*). The following subject inclusion and exclusion criteria define study population.

INCLUSION CRITERIA.

1) Men, ages 35 to 75 years.
2) Stable exertional angina pectoris.
   a) Duration of 3 months or more.
   b) Angina class II or greater (Canadian Cardiovascular Society Classification) (*15*).
3) A positive exercise treadmill test for myocardial ischemia, defined by:
   a) Exercise-induced ST-segment depression or elevation on the ECG compared with the rest tracing, as defined in the methods section (ST end point)
   b) Associated exercise-induced angina. (The presence of angina was required in order to address the outcome variable of time to onset of angina, which was used in prior studies.)
4) Documentation of the presence of coronary artery disease, demonstrated by one or more of the following criteria to provide additional objective evidence of coronary artery disease to exclude subjects with false-positive exercise studies:
   a) Angiographic evidence of coronary artery disease with one or more major coronary artery with diameter narrowing of 70% or more.
   b) Prior myocardial infarction, documented by at least two of the following:
      i) typical chest pain lasting 1 hr or more;
      ii) new Q waves enduring 0.04 sec or more, or T-wave inversions lasting longer than 1 week on the 12-lead ECG;
      iii) creatine kinase (serum) rise greater than twice normal with 10% or more MB isoenzyme fraction.
   c) Positive thallium stress test with unequivocal perfusion defect.
5) Ability and willingness of the subject to provide informed consent.
6) Permission of the subject's primary physician.
7) Stable anti-ischemic medical regimen for the study duration, or no medication. (Except for digoxin, exclusion criterion 7, there were no limitations regarding medical therapy other than that all medications were to be continued in unchanged doses and at standard dosing intervals throughout the study period.)
8) Ability to exercise for at least 3 min using the modified Naughton protocol, with reproducible total exercise duration on two tests performed at visit 1. (The reproducibility requirement demanded that the total exercise duration times be within 150 sec in order to exclude subjects with significant variations in serial exercise performance, in whom variant angina might be present and in whom the effect of CO exposures would be difficult to interpret.)

EXCLUSION CRITERIA.

1) Cigarette, cigar, or pipe smoking within 3 months (by history). (Current smokers were excluded because of chronic high COHb levels.)
2) Unstable angina, defined by new onset or change in pattern of angina within 3 months, or symptoms of angina at rest. (These patients were excluded because of their high risk and unpredictable clinical course.)
3) Myocardial infarction within the previous 3 months (3-month stability of symptoms as required in exclusion criterion 2).
4) Symptomatic congestive heart failure and congenital or significant valvular heart disease. (Excluded to minimize risk and to obtain a more homogeneous study population for repeated exercise testing.)
5) Previous coronary artery bypass surgery within 6 months. (Early postbypass patients were excluded because of complications of recovery.)
6) Resting ECG abnormalities that may interfere with ST-segment interpretation during exercise, defined by the exclusion of subjects with resting ECGs that meet the following Minnesota code classifications (codes in parentheses) (*16*): left ventricular hypertrophy (3-1, 3-2, 3-3, 3-4); ST depression (4-1, modified to read 2.0 mm); inverted T waves (5-1); A-V conduction defects (6-1, 6-2, 6-4, 6-8); interventricular conduction defects (7-1, 7-2, 7-4); tachyarrhythmias (8-2, 8-3, 8-4, 8-5, 8-6); and large amplitude T-waves (9-6).
7) Digoxin therapy or uncorrected hypokalemia.
8) Significant pulmonary disease defined by a forced expiratory volume in 1 sec (FEV_1) less than or equal to 50% predicted for age, height, and gender, or previously documented resting hypoxemia (arterial pO_2 less than or equal to 60 mm Hg), or oxygen saturation less than 90%. (The performance of arterial blood gas or ear oximetry was not required, but could be performed at the investigator's discretion if significant pulmonary disease was suspected.)
9) Uncontrolled hypertension (systolic pressure greater than 170 mm Hg or diastolic pressure

greater than 100 mm Hg at rest). (It was felt that items 8 and 9 potentially limited exercise performance significantly, and thus volunteers exhibiting either characteristic were excluded).
10) Anemia (hemoglobin less than 10 g/dL).
11) Thyrotoxicosis or other uncontrolled endocrine disease.
12) Symptomatic cerebrovascular disease, including recent stroke or symptoms suggestive of transient ischemic attacks.
13) Other significant debilitating systemic disease.
14) Inability or contraindication to perform exercise treadmill testing or to return for follow-up visits.
15) Initial venous COHb level at visit 1 greater than the exclusion criterion, as described under "Methods." (The CO oximetry exclusion criterion was chosen to represent 1% or less by GC.)
16) Presence of a permanent pacemaker.

Subject Recruitment Strategy. Subjects were recruited from the three test centers and included outpatients in the cardiology clinics, patients undergoing clinical exercise treadmill testing, patients scheduled for cardiac catheterization, and past and present participants in cardiac rehabilitation programs. Male subjects with positive exercise treadmill tests were further screened for smoking history, and potential candidates were then approached for visit-1 screening after permission from the subject's physician was obtained. Initial screening of subjects was performed by the study investigators with the assistance of research technicians and nurses.

Subject Enrollment and Exclusion. Seventy-six subjects, who reportedly met all visit-1 criteria, were enrolled in the study. Data from 63 of them were used in the main data analysis, reported in the results section of this report. The reasons for exclusion of subjects are summarized in Table 4.

Seven of the 76 enrolled subjects did not complete all test visits, leaving a total of 69 subjects who completed the three test visits. Of the 69 subjects who completed the four visits, six were excluded from the main data analysis because they did not meet all protocol criteria. Analysis of data from the 69 subjects is included in Appendix B. Data from 63 subjects were used in the main analysis of the effect of 3.9% COHb on time to angina, and data from 62 subjects were used in the analysis of the effect of 2.0% COHb on time to angina. Because one subject provided no ST data, there are only 62 and 61 subjects in the 3.9% and 2.0% COHb analyses of time to ST, respectively. The range in time from enrollment in the study to study completion was 10 to 35 days.

Table 4. Summary of subject exclusion from study and main data analysis.

76 subjects enrolled in study after completing visit 1
7 did not complete three test visits
69 subjects completed three test visits
1 disqualified on basis of visit-1 reproducibility requirement
4 disqualified on basis of visit-1 ST criteria
1 disqualified on basis of sample-1 COHb levels at two test visits
63 subjects provided data for angina analysis of 4% effect (62 subjects provided data for ST analysis of 4% effect because one subject did not provide ST-end point data)
1 excluded from 2% analysis because of sample-1 COHb levels
62 subjects provided data for angina analysis of 2% effect (61 subjects provided data for ST analysis of 2% effect because one subject did not provide ST-end point data)

Characteristics of Study Population. The characteristics of the study population are presented in Tables 5, 6, and 7. Table 5 describes the criteria met by each subject with respect to evidence of coronary artery disease. Table 6 presents information about general medical and cardiac history and medication use, as well as demographic information. Table 7 summarizes quantitative information on age, height, weight, and cardiac and pulmonary function.

Sixty-three male, nonsmoking subjects with a mean age of 62.1 ± 8.1 years, with stable angina pectoris and positive exercise treadmill tests that showed ischemic ST-segment changes, completed the protocol. In addition, as indicated in Table 5, each subject fulfilled at least one other criterion for coronary artery disease: angiographic evidence, prior myocardial infarction, or a positive thallium stress test. The majority of subjects was classified as having class II angina (*15*). Ten had prior coronary artery bypass surgery. Thirty-three subjects had a history of previous myocardial infarction; of this group, 26 had objective evidence of myocardial infarction, as defined by inclusion criterion 4. Twenty-seven subjects had a positive thallium perfusion scintigraphic study, and 40 had previous cardiac catheterization that demonstrated obstructive coronary artery disease. The mean number of anginal episodes per week at study entry was 4.6 (range of 0 to 63), and the mean number of nitroglycerin tablets taken per week was 2.3 (range of 0 to 30). Although 31 subjects reported a history of hypertension, no subject had uncontrolled hypertension during the study.

Coronary angiography had been performed in 43 subjects, although it was not a necessary prerequisite for study inclusion. Forty subjects had at least one vessel with 70% or more diameter obstruction by visual estimation. Thirteen subjects had single-vessel coronary artery disease, 15 had double-vessel coronary artery disease, and 12 had triple-vessel coronary disease. No subject had significant left main coronary disease.

Information on resting ECG abnormalities is summarized in Table 6. Although a significant number of subjects had resting P- and T-wave abnormalities typical for subjects with coronary artery disease, only a small number had minor resting ST-segment elevation (5) or ST-segment depression (7). In no case was the degree of resting ST-segment changes felt to be incompatible with the interpretation of ischemic ST-segment changes produced during exercise. Pulmonary function assessed by spirometry demonstrated a mean

Table 5. Criteria of coronary artery disease by which each subject qualified for the study.[a]

Subject	Coronary artery disease indicator: Angiography (≥ 70% lesion)	Myocardial infarction (objective evidence)	Positive thallium test
Johns Hopkins			
101	+		
102			+
103	+		
104	+	+	+
105	+		
106	+	+	
107	+	+	
108	+		
109		+	
110	+	+	+
111		+	
112	+		
113		+	
114	+		
115	+	+	+
116		+	
117	+	+	
118		+	
121		+	
122	+		
124	+	+	
125		+	
Subtotal	14	13	5
Rancho Los Amigos			
201	+	+	
205	+		
207	+	+	+
213	+		
214	+		
215	+	+	
218	+		
221			+
222	+	+	
227	+		
229	+		
Rancho Los Amigos (continued)			
234		+	
235			+
236	+		
237	+	+	
239			+
241			+
243		+	
Subtotal	12	7	5
St. Louis			
301	+		
303	+		
304	+	+	+
306			+
310	+		
311	+	+	+
317	+	+	+
318	+		+
323	+	+	+
324	+	+	
325			+
326	+	+	+
327	+		+
328	+		
330	+		
332			+
333	+		+
334			+
335			+
336			+
337			+
338			+
339			+
Subtotal	14	6	17
Total	40	26	27

[a] Any one of these three criteria was required for entry. In addition, each subject had stable exertional angina pectoris and a positive exercise treadmill test, as required by the protocol. (+) Indicates that the subject fulfilled the criterion. Information is not available to distinguish an absence of information from failure to meet the criterion.

FEV_1 of 88% of predicted values, and a mean forced vital capacity (FVC) of 90% of predicted values for age and size. Thus, significant obstructive or restrictive pulmonary disease was not found in the study population.

Although none of the subjects had smoked for at least 3 months prior to study entry, 51 had a history of past cigarette smoking. Forty-eight of these had stopped smoking more than 12 months before study entry, one had stopped 6 to 12 months prior to study entry, and two had stopped 3 to 6 months prior to study entry. The low COHb levels in venous blood samples upon arrival at the testing center provided evidence of compliance with the nonsmoking requirement. No subject who entered the study had to be excluded on the basis of suspected resumption of smoking during the study period.

Most subjects were receiving stable doses of beta-adrenergic blockers, nitrates, or calcium channel antagonists during the study period. The number of subjects using each type of medication is reported in Table 6. All subjects were carefully instructed to take their usual cardiovascular medications at the same time on the study days, and testing was scheduled at a standard time of the day for each subject to ensure a uniform time interval between the administration of cardiovascular medications and exercise testing.

All subjects underwent physical examination at study entry. As shown in Table 7, they had a mean weight of 83.0 ± 10.2 kg and a mean height of 176.1 ± 7.2 cm. Their mean resting heart rate was 63.3 ± 10.9 beats per minute. Mean systolic blood pressure was 131.2 ± 20.8 mm Hg (sitting), and diastolic blood pressure was 77.5 ± 8.6 mm Hg (sitting). No patient had evidence of congestive heart failure; no S3 gallops, rales, or evidence of elevated venous pressure were detected. An S4 gallop was detected in six subjects, three subjects had systolic murmurs, and one had a diastolic murmur; all of these murmurs were interpreted to be clinically insignificant. Three subjects had evidence of

Table 6. Baseline characteristics of the study population (63 subjects).

Characteristics	Number of subjects	Characteristics	Number of subjects
Heart disease		Medications	
Stable angina pectoris	63	Beta-blockers	38
Angina class II	60	Nitrates (oral)	30
Angina class III	3	(patch)	6
History of angina at rest	7	Calcium antagonists	40
History of myocardial infarction	33	Diuretics	13
Prior coronary artery bypass surgery	10	Antiarrhythmics	4
Information on coronary angiography	43	Antihypertensives	7
≥ 70% lesion	40	Other cardiac	7
Single-vessel CAD[a]	13		
Double-vessel CAD	15	Demographic information	
Triple-vessel CAD	12	Occupation	
Left main CAD	0	Professional	20
		Clerical	6
Resting electrocardiogram		Laborer	31
P-wave abnormality	9	Other	6
T-wave abnormality	21		
Anterior MI[b]	10	Employment status	
Inferior/posterior MI	11	Full time	25
Resting ST-segment elevation	5	Part time	3
Resting ST-segment depression	7	Not employed	4
Positive thallium stress test	27	*Disabled*	5
		Retired	26
Disease history			
Hypertension	31	Education	
Diabetes mellitus	10	Grade school	5
Cerebrovascular disease	4	High school	34
Peripheral vascular disease	2	Some college	15
Valvular heart disease	1	College graduate	6
Pulmonary disease	2	Graduate school	2
Thrombophlebitis	1	Unknown	1
Hepatic disease	1		
Renal disease	3	Marital status	
Gout	10	Single	5
Neoplastic disease	1	Married	50
Peptic ulcer	4	Divorced/separated	3
Hyperlipidemia	12	Widower	4
		Unknown	1
Smoking			
Currently nonsmoking	63	Religious affiliation	
Prior smoking (cigarettes)	51	Protestant	30
When stopped smoking		Catholic	19
3 to 6 months prior to study	2	Jewish	1
6 to 12 months prior to study	1	Other	11
More than 12 months prior to study	48	Unknown	2

[a] CAD, coronary artery disease.
[b] MI, myocardial infarction.

Table 7. Quantitative baseline subject characteristics.

Characteristics	Mean ± SD
Age	62.1 ± 8.1
Weight, kg	83.0 ± 10.2
Height, cm	176.1 ± 7.2
Angina duration, months	65.5 ± 64.8
Angina episodes/week	4.6 ± 9.4
Nitroglycerin tablets/week	2.3 ± 5.5
Resting heart rate, beats/min	63.3 ± 10.9
Blood pressure, mm Hg	
Systolic (sitting)	131.2 ± 20.8
Diastolic (sitting)	77.5 ± 8.6
Systolic (standing)	130.4 ± 17.9
Diastolic (standing)	78.1 ± 9.4
Spirometry	
FEV_1, L	2.8 ± 0.5
FEV_1, % predicted	88.3 ± 18.3
FVC, L	3.7 ± 0.7
FVC, % predicted	90.0 ± 15.7

cardiac enlargement and four had abnormalities of lung fields on chest X-ray examination.

Although the major clinical characteristics were comparable at the three testing centers, several minor differences were noted. There was a lower percentage of subjects in professional occupations enrolled at Johns Hopkins University (9%) than at St. Louis University (39%) or Rancho Los Amigos Medical Center (50%). There were minor differences in measured electrolyte values, including sodium, potassium, and chloride, but these values were within normal limits at each center.

Carbon Monoxide Exposure and Monitoring

Carbon Monoxide Exposure Methods. The pro-

cedures used for exposing the subjects to CO were intended to result in end-of-exercise COHb values of 2.0 or 4.0% as determined by GC. To attain these levels of COHb in the subject's blood, the target levels after the exposure were set at values 10% greater than the desired end-of-exercise levels, that is, 2.2 and 4.4% COHb. Subjects exercised while they breathed room air that contained no more than 8 ppm CO, which resulted in the elimination of some of the body burden of CO taken up during the exposure phase. The subjects at all three centers were exposed in room-sized chambers (700 to 2000 ft^3) for approximately 1 hr. The chamber concentrations were adjusted for each subject based on his CO-uptake data obtained at visit 1, but were fixed for the duration of each exposure. Due to variability in a given subject's CO-uptake rate, the length of each exposure was also varied, with the goal of attaining end-of-exposure levels of COHb as close to the target levels as possible. The effectiveness of the exposure protocol in narrowing the range of COHb values is discussed in Appendix C.

Each individual's CO-uptake rate was determined at the end of the qualifying visit to the laboratory (visit 1). The subjects were exposed to 150 ppm CO for 1 hr, and blood samples were drawn prior to and every 15 min during the exposure period. These samples were analyzed immediately by CO oximetry for %COHb. The %COHb values were plotted against duration of exposure, and the 60-min uptake rate was computed based upon a linear regression of the slope, with the units of change in %COHb per hour. This value was divided by the actual level of CO to which the subject was exposed (approximately 150 ppm), resulting in an uptake-rate constant expressed as change in %COHb per hour per ppm CO.

On the randomized test visits, the level of CO in the chamber required to increase the %COHb from the sample 2 (preexposure) level to the target level was computed by the following equation: CO concentration (ppm) = [(target %COHb) − (sample 2 %COHb)]/(uptake rate constant).

Since the uptake-rate constant was determined on the basis of only one exposure, allowance for variability in the uptake rate on subsequent visits was provided for by measuring uptake at 30 and 40 min and varying the length of exposure from 50 to 70 min. The appropriate length of exposure to CO was established graphically by linear extrapolation from a plot of the COHb levels at 0, 30, and 40 min (Fig. 4).

To minimize learning or training effects on the study results, the order of exposure among the three possible conditions of exposure was randomized (Appendix A). The Statistical and Data Management Center assigned a randomization order for each subject as he qualified for entrance into the study (successful completion of visit 1). The effectiveness of the randomization process for determining the order of exposure to air, 2% COHb target CO level, and 4%-COHb target CO level is summarized in Table 8. A comparable number of subjects received each of the three exposures on each of the testing days.

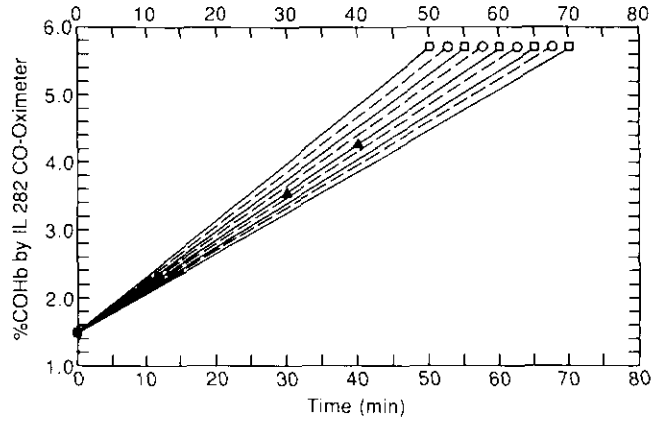

FIGURE 4. Determination of length of exposure for a given subject. The GC target is 4.4% COHb, corresponding to a CO oximeter target of 5.7%. The preexposure sample was analyzed and plotted at time 0. This point was connected to the possible end-of-exposure times of 50, 55, 60, 65, or 70 min (open square and solid lines). Additional decision lines were also drawn at 52.5, 57.5, 62.5, and 68.5 min and the initial data point (open circle and dashed lines). The values of COHb obtained at 30 and 40 min were then plotted on this graph (filled triangle). Since these data points fell between the 57.5- and 62.5-min decision lines, the exposure was carried out for 60 min.

Table 8. Results of randomization of order of blinded exposures to purified air, carbon monoxide to 2%-COHb target, and carbon monoxide to 4%-COHb target.

	Number of subjects		
Exposure	Visit 2	Visit 3	Visit 4
Combined			
Air	20	24	19
2% COHb	20	22	20
4% COHb	23	16	24
Johns Hopkins			
Air	7	8	7
2% COHb	8	8	6
4% COHb	7	6	9
Rancho Los Amigos			
Air	5	7	6
2% COHb	4	7	7
4% COHb	9	4	5
St. Louis			
Air	8	9	6
2% COHb	8	7	7
4% COHb	7	6	10

The CO oximeter provided a method of rapidly analyzing blood samples for %COHb, as was required to monitor the CO exposures. The exposure levels were set in an attempt to reach COHb levels as measured by GC. Because %COHb values obtained by GC and CO oximetry differ, an average offset was used to determine CO oximetry target values. For most of the study,

Table 9. Individual center results for COHb levels in venous blood samples determined by CO oximeter.[a]

Center	Air day			2%-COHb-target day			4%-COHb-target day		
	n	%COHb	SEM[b]	*n*	%COHb	SEM	*n*	%COHb	SEM
After exercise test 1 (sample 2)									
Johns Hopkins	22	1.13	0.07	22	1.18	0.07	22	1.12	0.05
Rancho Los Amigos	18	1.25	0.09	18	1.18	0.09	18	1.22	0.08
St. Louis	23	1.35	0.09	22	1.35	0.08	23	1.35	0.08
After exposure (sample 5)									
Johns Hopkins	22	1.25	0.06	22	3.33	0.06	22	5.76	0.08
Rancho Los Amigos	18	1.34	0.07	18	3.13	0.07	18	5.56	0.13
St. Louis	23	1.54	0.08	22	3.15	0.06	23	5.42	0.09
After exercise test 2 (sample 6)									
Johns Hopkins	22	1.07	0.07	22	2.78	0.07	22	4.79	0.07
Rancho Los Amigos	18	1.13	0.08	18	2.58	0.07	18	4.76	0.10
St. Louis	23	1.26	0.09	22	2.56	0.05	23	4.55	0.10

[a] Measurements were made on samples taken at the end of exercise test 1, at the end of the exposure period, and at the end of exercise test 2.
[b] SEM, standard error of the mean.

an offset of 1.0% was used for the 2.2% GC target and 1.3% for the 4.4% GC target, giving CO oximeter target levels of 3.2 and 5.7%, respectively. The ability to attain the CO oximeter target values is a validation of this technique (Table 9).

Monitoring of Atmospheric Carbon Monoxide. The levels of CO breathed by the subjects in this study were measured at all stages of the experiments. This included measuring the background laboratory CO levels to which the subjects were exposed during the exercise tests as well as the chamber CO concentrations during the exposure phase. Carbon monoxide levels were determined with the use of nondispersive infrared analyzers (Bendix Model 3501-5CA, or Beckman Model 866) that provided a continuous recording of the CO concentration. Prior to use each day, the instruments were calibrated with commercially available standard gases that had been analyzed by the suppliers (Airco, Detroit, MI, or Scott Specialty Gases, San Bernardino, CA) and were shown to be ± 1% of the reported concentration based upon National Institute of Standards and Technology (NIST) standards. In addition, cylinders of CO that had been standardized according to the EPA protocol (*4*) were used for daily calibration and to confirm periodically the concentration of the NIST traceable gases. The EPA traceable gases not only met the requirements for the NIST gases, but also had to show consistent values (within 1%) on repeated analyses at least 24 hr apart. All of these gases were contained in aluminum cylinders treated with an antioxidant to prevent production of CO in the cylinders over time. The zero-CO gas mixtures used were nitrogen without carbon dioxide for the Bendix instruments and nitrogen with 0.03% carbon dioxide for the Beckman analyzer.

The daily calibration of the analyzers was carried out using two or three standard gases for each range of expected use of the instrument on CO-exposure days. On air-exposure days, the analyzers were calibrated with at least a zero-gas mixture and one standard gas. The gases were monitored until a stable reading was maintained for at least 1 min on the chart recording.

Five times over the 2-year course of testing of subjects, the Rancho Los Amigos center coordinated a round-robin analysis at all three centers of cylinders with two unknown concentrations of CO specially prepared each time for this task. The results of these comparative tests provided information about the relative values for monitoring atmospheric levels of CO at all centers and about the functioning of the instruments that were used throughout the course of the study. These results indicate that all centers were within 2% of each other with regard to the range of values and within 1% when the average difference was used.

A more extensive calibration of the analyzers was carried out periodically. During these sessions, the standard gases were calibrated against the EPA protocol gases over all the intended ranges of use of these instruments. It was the general finding that none of the gases varied from one another, regardless of the protocol under which they were produced at the supplier. Also, the gases remained stable for the 2.5 years that they were used for this study.

The monitoring during exposure at each center was done in such a way that the gas that was sampled reflected the CO concentration that the subject was inhaling. This was carried out by sampling next to the head of the subject (within 1 to 2 ft) at Johns Hopkins and St. Louis. At Rancho Los Amigos, sampling was at a distant site, and the distribution of CO and the mixing characteristics of the chamber were checked every 3 months to confirm that there were no gradients of CO in the chamber. Similar checks of chamber mixing were also performed at Johns Hopkins and St. Louis, at less frequent intervals.

Atmospheric levels were continuously monitored during exercise testing and never exceeded 8 ppm. During the exposure period, a mean or integrated value of the atmospheric CO concentration was reported to the nearest part per million. The longest in-

terval from which averages were computed was 15 min.

All subjects were exposed to the various levels of CO in room-sized chambers equipped with CO monitors and with temperature and humidity controls. The temperature during exposure ranged from 65° to 70°F, and the relative humidity ranged from 45 to 65%. The air volume in these rooms was rapidly turned over for atmospheric control; the exchange rate varied from 15 to 50 times per hour. During the exposures, the seated subjects could not see the chamber operator or gas monitors. The subjects entered the chambers only after the desired level of CO was achieved.

Carboxyhemoglobin Measurements

Gas Chromatography. Other studies that investigated the influence of low doses of CO on the exercise tolerance of patients with coronary artery disease used spectrophotometric techniques for assessing blood levels of CO (*5–7,17–19*). Spectrophotometric methods are not sufficiently sensitive for assessing very low levels of COHb. The error of analysis for the IL 282 CO oximeter is reported to be 1.0% COHb (*20*), which is greater than the expected resting COHb level in nonsmokers. Therefore, for this study, GC, a more accurate technique for the measurement of CO in blood (*4*), was used to determine COHb levels. The advantage of the GC method used in this study is that it is precise (resolution better than 0.01 ml of CO/dL of blood) and linear throughout the entire range of potential values of CO (up to 100% COHb), without modifying the analytical technique (*21*). Also, the GC results from these samples are unaffected by all the factors that may influence the CO oximeter values.

The CO content of each sample of venous blood was measured by GC by the Dahms and Horvath (*21*) technique. By this technique, the CO contained in 200 μL of blood is released from the hemoglobin by denaturation of the hemoglobin and is then extracted into the headspace of the sealed reaction vial with the use of a vortex. The headspace containing the CO is eluted onto the GC columns. The columns used for separating the CO from the other gases were Porapak Q and molecular sieve in series. The CO distribution curves were measured by thermal conductivity sensors; peak height was used for quantification. The system was calibrated by adding known amounts of CO in place of the blood in a standard reaction vial. All values were corrected to standard temperature and pressure, dry (STPD) conditions.

Gas chromatographic analysis provided a value for the CO content of each sample. The CO content was converted to %COHb by using a calculated CO capacity derived from triplicate measurements of hemoglobin by the cyanmethemoglobin technique. The capacity was calculated by the formula capacity = average hemoglobin (g/dL) × 1.389 mL/g (*22*).

The binding capacity was verified on a monthly basis. Samples of fresh whole blood were saturated with CO by equilibration for 10 min with a gas mixture of 99.9% nitrogen and 0.1% CO. This last step reduced the amount of dissolved CO (unbound to hemoglobin) to negligible levels, while maintaining saturation of the hemoglobin with CO. The analysis of these saturated samples by GC provided a measurement of the CO capacity that could be compared to a capacity calculated on the hemoglobin values for these same samples.

Care was taken in the determination of hemoglobin using the cyanmethemoglobin technique in those specimens containing high levels of COHb: The full development of color required 4 to 8 hr. This was determined using a Beckman model spectrophotometer, with measurements every 10 min for a 12-hr period. These specimens were pipetted, sealed, and stored in the dark overnight prior to analysis. The measured value averaged 98% of the expected value; the range of values varied between 93 and 102%. Some of this difference could have been due to the presence of methemoglobin, which was not measured. The agreement between the techniques provided the necessary internal validation of the cyanmethemoglobin technique.

Rather than establish this GC technique at three separate locations, samples requiring GC analysis were shipped to the Reference Laboratory in St. Louis for analysis. This was possible because COHb is very stable in a well-sealed syringe. Even though these samples would remain stable for weeks, they all were sent to the reference laboratory within 3 days of collection and analyzed within 3 days of receipt.

All samples were analyzed in triplicate or until three results were attained that were within a range of 5% of the average value or 0.02 mL/dL of blood. The values used in all the data analyses from this study represent the mean of the three values for each sample. Hemoglobin determinations were made on each sample. If the samples yielded inconsistent results, they were hemolyzed with dry saponin and reanalyzed. Hemolysis with saponin was shown to have no effect on the CO content of blood samples or on the hemoglobin content, as measured by the cyanmethemoglobin technique.

Three blood samples from each test visit (visits 2, 3, and 4) for each subject were analyzed by GC: the sample taken immediately after the first exercise test (sample 2), the sample collected at the end of the exposure period (sample 5), and the sample collected at the end of the second exercise test (sample 6). The samples at the end of each exercise test were those representing the COHb levels at approximately the time the indicators of myocardial ischemia were measured.

CO Oximetry. The optical method employed by CO oximeters provides a means of obtaining approximate measurements of COHb levels rapidly. The IL 282 CO oximeter (*20*) was used in this study because of the need for immediate information on blood COHb levels and the extensive use of this instrument in this area of research by other investigators. Rapid measurements were needed to determine if subjects' baseline COHb levels were below the cut-off level and to determine the duration of the CO-exposure period. These were sam-

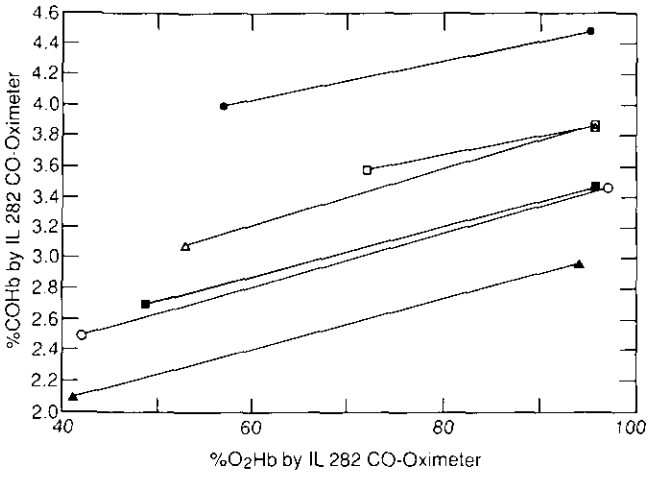

FIGURE 5. Effect of oxygenating several samples from the same subject during a CO-exposure protocol (increasing initial COHb values). Note the consistency in the slope of the lines. Also note the variability in the initial $\%O_2Hb$ values.

ples 1, 3, and 4 during the three test visits, as shown in Table 2. Other samples, which were measured by GC, were also measured by CO oximeter.

Four IL 282 CO oximeters were used in this study: one at each test center and one at the reference laboratory. The standard operating procedure was as described in the IL 282 manual (*20*). The instrument operation was checked at the beginning of each work day, with a control dye solution obtained from a commercial vendor. The dye solution was analyzed to check the calibration, and the instrument was adjusted, if necessary, for hemoglobin concentration.

Considerable effort was made to investigate the factors affecting CO oximeter measurements of %COHb, with the goal of improving the accuracy of measurements and the comparability of values among individuals. Of the many factors investigated, the O_2Hb content of the blood was found to have a major effect on the CO oximeter values for %COHb (Fig. 5).

The relative operation of the instruments at the three centers was compared with the use of modified whole human blood standards that were prepared specifically for this purpose. A method was developed by the reference laboratory for producing a set of fresh, whole-blood samples with known amounts of %COHb determined by GC (T. E. Dahms, in preparation).

Throughout the study, a set of four blood samples, containing 0.5% to 8% COHb, was provided on ice to the three test centers every 2 weeks. The first day of analysis at each laboratory was the day following shipment. The reference laboratory carried out daily analyses on the samples for 3 days, starting with the day of shipment. This enabled same-day comparison of data collected on these standards, in case the standards showed a change in their optical characteristics that could result in a change in the value for %COHb. The reference laboratory included in the shipment with the samples the data from the day-1 analysis by both GC and CO oximetry. This enabled the receiving laboratory to identify any problem with its instrument.

These blood standards were not intended to serve as a means of calibration of the instruments, but to provide a means for comparing measurements by the instruments at each of the three test centers and for identifying problems with the instruments. No attempt was made to standardize the readings among centers. The results obtained on these standard sample sets were transmitted to the Statistical and Data Management Center for monitoring and analysis. The results were also transmitted back to the reference laboratory for the purpose of monitoring the performance of the instruments.

Exercise Treadmill Testing

General Considerations. After meeting the inclusion/exclusion criteria, the subject was scheduled for the chamber exposure and exercise-test protocol. All subjects were screened by one of the cardiologists prior to randomization. Once the subject was entered, the entry was considered final and the subject was not removed from the study at a subsequent date except for the specified criteria described below.

There was no CO exposure on the first exercise test on all four visits. This provided individual reproducibility data for exercise end points for each subject. A pretest interim history and 12-lead ECG were done at each visit to verify that the angina and ischemic heart disease had been stable. If there was evidence of unstable angina, the subject was excluded from the study.

A venous cannula was inserted for the duration of each day's testing, and a pretest COHb level was determined to document the lack of recent exposure to significant levels of CO. If the COHb level was above the cut-off level, the subject's visit was cancelled for the day and rescheduled. After the February 1986 protocol amendment, if the subject failed to develop angina or an ischemic ST change on the first test of any visit, the visit was rescheduled and the exposure and second test for that day were not done. Furthermore, if the subject failed to meet either the angina or ST-change requirement or the COHb inclusion criteria on two successive visits, he was dropped from the study.

The Mason-Likar (*23*) preexercise supine, sitting, standing, and hyperventilation 12-lead ECGs were used to determine if posture- or hyperventilation-induced ST-T-wave changes occurred. The control ECG recorded at each visit, along with an interim history, was used to assure that no new coronary event had transpired since the previous test. The preexercise standing 12-lead Mason-Likar ECG (including -AVR) was the baseline reference for all the ST-segment end point measurements for that day's tests.

Specific Definitions. ECG ST-SEGMENT RESPONSE. There are three types of ST changes in the ECG that are interpreted as being indicative of myocardial is-

chemia, referred to collectively in the text as "ST end point":

Type 1. J-point elevation ≥ 1.0 mm and a horizontal or upsloping ST segment 80 msec after the J point in a lead in which abnormal Q waves were not evident;

Type 2. J-point depression ≥ 1.0 mm and a horizontal or downsloping ST segment 80 msec after the J point;

Type 3. J-point depression ≥ 1.5 mm and an upsloping but depressed ST segment 1.5 mm below baseline 80 msec after the J point (the end point in 34 tests). The slow, upsloping ST segment depression has been reported to indicate myocardial ischemia in patients with documented coronary artery disease (*24,25*).

ANGINAL CHEST PAIN. The subject was instructed to report any symptoms to the investigator and to grade his chest pain on a scale of 1 to 4 as follows: *a*) L-1: onset of angina, mild, but recognized as the usual angina-of-effort pain or discomfort with which the subject is familiar. *b*) L-2: same pain; moderately severe, definitely uncomfortable, but still tolerable. *c*) L-3: severe anginal pain, at a level that the subject will wish to stop exercising. *d*) L-4: unbearable chest pain, the most severe pain that the subject has felt.

ST AND ANGINA END POINTS. Time to reach the earliest typical qualifying ST change, measured in seconds from the onset of exercise, was determined. The lead from the visit-1 preexposure tests in which this occurred was entered as the designated lead, along with the type of ST change (1, 2, or 3). The lead and type of ST change generally remained the same in any given subject for all exercise tests, on both qualifying and test visits. In the unusual instance in which the time to onset of a different type of ST response occurred earlier in any lead in a subsequent test, the lead and the type of response were recorded as the time to ST depression or elevation only if this ST response occurred 2 min or more before the usual ST response in the designated preexposure lead.

Type 1 changes were the end point in two exercise tests, type 2 in 455, and type 3 in 34. In 57 subjects, the same type of ST response was used in all exercise tests. In five subjects, both type 2 and type 3 changes were used; in one subject type 1 and type 2 changes were used. In fifty-nine subjects, the same leads were used for the ST end point in all exercise tests. In four subjects, 2 leads were used; in two of these subjects, V5 and V6 were used; in one, V4 and V5 were used; and in one, V5 and II were used.

The duration of the ST change, in seconds, in recovery was also recorded for the designated lead. Similarly, time to the onset of angina, measured in seconds, from the beginning of exercise to the onset of the earliest recognized angina (level 1) was determined, along with the duration of angina.

Exercise Testing. The exercise treadmill test was performed on a standard motorized programmable treadmill, using the modified Naughton protocol (*12*), as shown in Table 10. Subjects exercised for 2 min at each stage of the protocol; each stage was designed to increase the workload by an estimated 1 MET (basal metabolic equivalent). The maximum workload on the protocol was 11 METs at 18 to 20 min. The scale readings for treadmill speed and grade on the electronic programmer were verified on a regular basis by manual measurement of actual treadmill speed and grade. The ECG recording and digitizing system was calibrated before each recording session.

The 12-lead Mason-Likar ECG (including -AVR) was recorded throughout the exercise with a commercially available system (CASE II, Marquette Electronics, Minneapolis, MN) at Johns Hopkins and St. Louis, or with an emulation of this system on a General Automation minicomputer system at Rancho Los Amigos. Selected leads were monitored continuously for rhythm changes by oscilloscope, hard-copy analog tracings, or both throughout the testing procedure. The Mason-Likar 12-lead ECG was digitally sampled at 250 Hz or higher through buffered inputs and with sample and hold amplifiers that had a frequency response of from 0.05 Hz to at least 120 Hz, with a common mode rejection of 100,000 to 1 or better.

The signal processing consisted of a learning period when a typical cycle was selected (for all 12 leads). A template-matching algorithm was then applied to

Table 10. Modified Naughton protocol for exercise treadmill ECG test.

Stage	2.0-mph grade, %	3.0-mph grade, %	Duration, min	Estimated METs, units[a]	Elapsed time, min
1	0.0		2	2.0	2
2		0.0	2	3.0	4
3		2.5	2	4.0	6
4		5.0	2	5.0	8
5		7.5	2	6.0	10
6		10.0	2	7.0	12
7		12.5	2	8.0	14
8		15.0	2	9.0	16
9		17.5	2	10.0	18
10		20.0	2	11.0	20

[a] MET, resting metabolic equivalent.

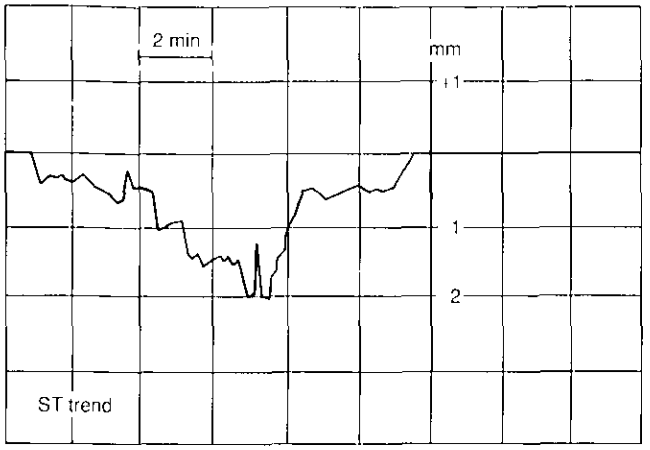

FIGURE 6. Trend plot of ST-80 measurements for the lead showing the worst ST-segment changes. The time to onset of ST end point was measured from this type of plot. Note that maximal ST displacement is 2 mm.

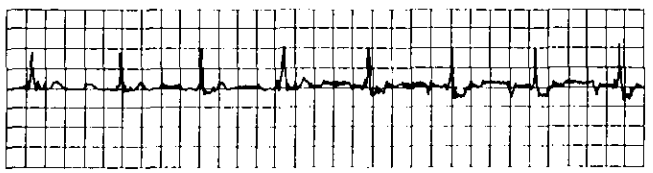

FIGURE 7. For the lead showing the worst ST-segment depression, this is the P-QRS-T-wave complex at baseline (first complex) and each minute thereafter. The three reference points shown with each complex represent the PQ junction, J point, and ST-80 measurement (also see Fig. 1).

each of the new 12 ECG incoming signals, with time alignment around a fiducial multilead QRS detector trigger. A running updated complex was generated that included P-QRS-T. The QRS onset and offset (J point) were determined automatically by the program and were displayed for verification. The amplitude of the ST 80 ms after J in the updated beat was plotted every 3 sec and was recorded as a hard-copy trend plot for each lead (Fig. 6). In addition, the amplitude of the ST 80 ms was printed out in digital form each minute (Fig. 7), along with heart rate and the processed 12-lead waveforms of the ECG. The time, to the nearest 10 sec, from the start of exercise to the onset of ischemic ST, was determined from the trend plots. A template-matching cycle selection and running averaged beat were processed with a continuous measurement and hard-copy plot of ST at 80 ms after the J point. Hard copy of the unprocessed and processed ECG was made at a minimum of 1-min intervals. Both the unprocessed and processed ECGs were manually overread, in order to verify the appropriateness of the cycle selection and signal processing of the ECG, the computer-generated QRS offset (J point), and the ST 80 measurements and trend plots.

Table 11. Number of times exercise was terminated for various reasons in a total of 376 exercise tests.

Reason for termination	Number of times
Fatigue	79
Angina criterion	306
Falling blood pressure	5
ST > 3 mm	18
Hypertensive blood pressure	6
Subject request	172
Other	42

The criteria for stopping the symptom-limited exercise tests were: *a*) fatigue, shortness of breath, leg pain, or subject's request to stop; *b*) level 2 angina approaching level 3 and subject wishing to stop; *c*) 3-mm ST-segment shift; *d*) hypertensive blood-pressure response (240 systolic or 130 diastolic); *e*) drop in blood pressure equal to or greater than 20 mm below peak, confirmed by a second reading within 20 sec (an elevated resting blood pressure, usually with anxiety, that stabilized without symptoms after an initial drop in the first 1 to 2 min of exercise was disregarded); *f*) significant dysrhythmia, that is, supraventricular tachycardia lasting more than 10 sec, multifocal preventricular contractions (more than 6 per minute) if associated with increasing angina or significant ST change, three or more preventricular contractions in a row, or second- or third-degree AV block; *g*) symptoms of possible central nervous system dysfunction, such as lightheadedness, dizziness, ataxia, nausea, and pallor; and *h*) equipment failure or poor ECG recording that prevented accurate interpretation of rate, rhythm, or ST change.

The reasons for termination in 376 exercise tests are presented in Table 11. Frequently, there was more than one reason for ending the test. Neither central nervous system dysfunction nor arrhythmia were ever a cause for termination.

Blinded Interpretation and Consensus Review of Electrocardiograms. The cardiologist at each center who reviewed the unprocessed and computer-processed ECG records was blinded to name, date, and exposure history for test visits. The tracings were analyzed in a batch; all qualifying tests and tests of visits 2 through 4 were reviewed at one time. Visit 1, in which the reproducibility requirement was addressed, was read in an unblinded fashion. All of the exercise ECGs (raw tracings and computer-processed records) for each subject were reviewed by the cardiologists from at least two centers. These reviews were done at several consensus meetings that occurred during the course of the study. The following end points were reviewed at these working sessions: time of onset of an ischemic ST response, as determined by review of the computer-generated ST trend plots and processed ECGs and confirmed by comparison with the raw data; duration of the ischemic ST change in the recovery phase; total number of leads exhibiting an ischemic ST response; and four coded maximum ST change and slope, during exercise and recovery, in 12 leads (including AVR).

Since the computerized digital trend plot of ST 80

was used to define the time to ST end point, the cardiologists' main role in ECG overreading was to evaluate the trend plot for stability and accuracy, to evaluate the ST-80 slope, and to define the type of ST response. The raw data were compared to the digitized computer-processed data at each minute of exercise to confirm that the signal-processed data conformed to the original. The main concern was to verify that no major artifact had been included in the processed data. It was determined that the noise reduction was such that a more reliable estimate of ST-80 amplitude and slope could be made from the computer-processed waveforms. Once confirmed by review of the raw data, the processed waveforms were used throughout the study to define the type of ischemic ST, to confirm the time to onset of ischemic ST to the nearest minute, and to code the maximal ST change and slope in each of the 12 leads.

The trend plots of ST-80 amplitude were then overread to refine the time to ischemic ST to the nearest 10 sec. This time was agreed on by consensus in each case. The coded amplitude of the maximal ST 80 and the type of ischemic ST in each of the 12 ECG leads (including -AVR) were also reviewed at these meetings. The slope of the ST 80 was overread as follows: using a 5× magnifying lens with an etched 1-mm scale at the focal plane of the magnifier, the baseline of the 1-mm scale was aligned with the ST segment just prior to and at ST 80. The slope of ST 80 was defined as flat if the slope of this portion of the ST was equal to ± 0.2. It was called upsloping when the slope of this portion of the ST was greater than 0.2, and downsloping if less than -0.2. Consensus was reached in each case.

Double-Blind Conditions

The double-blind conditions of this study were maintained in a rigorous fashion. All personnel involved with the study were instructed to maintain these conditions and were monitored by the investigators to assure compliance with this requirement. At each of the three centers, the exercise-cardiology personnel were kept blinded to the exposure conditions, but the exposure personnel were not. Communication between the two groups was restricted to issues of timing of collection of blood samples and duration of exposure. At no time were the chamber conditions discussed with the subject or with the exercise-cardiology personnel.

The exposure personnel were instructed and monitored to assure consistent behavior patterns on all randomized days to prevent their giving any subtle indication to the subjects or other personnel about the exposure conditions. The length of exposure on the air day was randomized in advance, but the end of the exposure was not announced until sufficient time had elapsed for analysis of the 40-min blood sample and calculation of the exposure time. The chamber monitoring, blood-sample analysis, and exposure-time calculation were performed out of sight and hearing of the exercise-cardiology personnel. The exercise-cardiology groups remained blinded through the data analysis phase of the study.

Data Collection and Management

Staff from the Statistical and Data Management Center worked closely with the investigators to design the data-collection forms. The forms were intended to serve as a complete record of each subject's eligibility for the study, medical history, laboratory history, CO exposures, exercise treadmill tests, and progress through the study from randomization to completion of the protocol.

Subjects who met the study inclusion criteria were randomized to exposure orderings after the qualifying visit. Randomization codes were issued by the staff of the Statistical and Data Management Center to the data coordinators at the clinical centers. The randomization plan called for stratification by institution and by whether or not the subjects had a previous myocardial infarction (MI). Within each of these six strata (3 institutions × 2 previous MI statuses), the subjects were balanced in groups of six so that each of the six possible orders of exposure (air, 2%-COHb target, 4%-COHb target) was represented.

Data were entered into a VAX 11/780 computer (Digital Equipment Corporation) running the UNIX operating system (Bell Laboratories and University of California, Berkeley) at Harvard Health Sciences Computing Facility, Boston, MA. The data-entry program performed range-checking on individual items and some cross-validation as data were entered. The data files that resulted from data entry corresponded to the data collection forms and were processed (entered, verified, and queried) in weekly batches corresponding with the data submission schedule.

Within a few days of receipt, the weekly data batches were entered into the computer. To ensure absolute accuracy in this data base, data listings were produced and visually checked against the data forms. Entry errors were then corrected in the data base. Logical errors and errors that showed up in cross-validation were queried at the clinical centers using a special query form, and these errors too were corrected in the data base. Data that passed the checking routine and data that passed after the query routine were advanced to a final data base for data analysis.

Analysis files were constructed from the data case and were maintained on-line on the VAX 11/780 computer, the IBM 4341 (IBM Corporation), or both during data-analysis activity. Magnetic-tape copies of all analysis files were made. Data were analyzed periodically throughout the study, but the results were not revealed to the clinical investigators until testing was completed.

Statistical Analysis

The goal of the primary statistical analysis was to determine whether or not there is an effect on the subjects in the study when they are exposed to CO as com-

pared to air, as measured by the time to ST end point (type 1, 2, or 3 ischemic ST-segment changes in the ECG) and the time to angina. The measurements were obtained on 3 separate days. On each day, there was a preexposure exercise test to obtain baseline measurements and a second exercise test after exposure in the chamber. Depending on the day, subjects were exposed to air without elevated CO levels or to CO levels designed to achieve approximately 2% COHb or approximately 4% COHb at the end of exercise. The measurements of both time to onset of angina and time to ST end point were compared to their respective baseline data to obtain the increase or decrease due to the exposure. Finally, the air exposure (no CO added) was used as a baseline measurement to get the four values for each subject: decrease in percent at 2% COHb and 4% COHb for the time to onset of ST end point and the time to angina (as specified in the protocol).

The 2% COHb and 4% COHb analyses are reported separately. Furthermore, separate analyses are also given for the three centers. To guard against outliers, trimmed means were used as summary statistics, with the two largest and two smallest observations trimmed (*26*), as specified in the protocol.

All significance calculations in the primary analysis used a one-sided p-value with significance at 0.05, looking only for a significant decrease in time based on permutation distribution of the trimmed means (*27*). The one-sided confidence interval was specified in the protocol because there was no basis to postulate that CO would have a beneficial effect on the development of ischemia (*28,29*). Since we were concerned with determining whether or not there is a significant decrease from zero, we considered all possible ways of assigning a plus or minus sign to every observation and calculated the resulting trimmed means. With m observations, there are thus 2^m points in the sample space, each point representing an onset of angina trimmed mean and an ST-segment-change trimmed mean. The significance of the observed statistic was then evaluated by considering all 2^m points as equally likely. The above analysis strategy was planned prior to any subject enrollment (and data collection) and is robust to outliers and model selection, yet it is efficient. Results of alternative analyses are presented in Appendix B.

Quality Assurance Procedures

Quality assurance (QA) procedures applied to this study were defined in the QA Plan developed and implemented during the planning stage of the study. The overall goal of the QA Plan was to assure that the study was carried out in a manner that produced data of high and well-documented quality, the conduct of the study was consistent among the centers, and the resultant data were of equal reliability. An independent QA Officer was responsible for overseeing the implementation of the QA Plan and for monitoring compliance with the QA Plan as the study progressed.

As required by the HEI QA Plan, the procedures used to conduct the study were defined in the experimental protocol, the standard operating procedures, and the DATA Management Procedures Manual developed for and applied to the study. Each of these documents was developed by the investigators and was reviewed and approved by HEI, the investigators, and the QA Officer. The protocol defined the purpose and background of the study, the expected results, the significance of the expected results, and the overall organization of the study team. It provided an outline of the methodologies to be used and a description of the data to be collected. Standard operating procedures documented all routine and critical experimental procedures and techniques. They provided specific information on calibration and maintenance of instruments, quality control, sample handling procedures, and the specific methodologies used. The Data Management Procedures Manual included standardized forms for the collection and reporting of data. It provided specific direction on the use of the data collection forms, the flow of data between the investigators and the Statistical and Data Management Center, the entry of data into the computer, including verification and storage procedures, and the primary statistical methods to be used to analyze the data. Standard operating procedures, data management procedures, and data collection forms were prepared in document-control format, with each having a unique title and identification number.

Changes to the experimental protocol were documented as protocol amendments to assure that all study team members had the information needed to implement the changes and that the changes were uniformly applied at each study center. The amendments detailed changes to be made, the reasons for making the changes, and the effective dates of the changes. Each amendment was approved by the principal investigators, the QA Officer, and the HEI project manager. The standard operating and data-management procedures were revised, as needed, to correspond with the amended protocol. Inadvertent or one-time-only deviations from standard operating procedures and the protocol have been documented in the study files at the test centers. A historical file of protocol amendments, standard operating procedures, data-management procedures, and data collection forms was maintained to ensure that there was a complete record of all the procedures used.

Facilities inspections were conducted at each study center by the QA team to determine whether the physical facilities were adequate for effective completion of the study. Prestudy on-site inspections at each center were used to document formally the qualifications and experience of the personnel, their familiarity with the protocol and methods, and the adequacy of the testing facility. Laboratory inspections, conducted at approximately 6-month intervals while the study was in progress, ensured that the quality and integrity of the data met the requirements defined by the QA Plan. These inspections included monitoring of the actual

conduct of the study, data audits, review of data collection and management procedures, and discussion and review of the QA Plan with the investigators. The data audit compared the raw data and the reported results to verify the validity of the final report. A primary concern of these audits was the determination that clear data trails exist that demonstrate that the study was conducted as defined in the protocol, standard operating procedures, and Data Management Procedures Manual. A confidential inspection report was prepared for HEI's Executive Director at the conclusion of each audit. HEI's Executive Director gave each report to the project manager for the study; she transmittted it to the appropriate principal investigator. The report detailed any significant findings and requirements for corrective action.

The QA group conducted the final quality assurance audit on the data at the end of the study. A report by the QA officer states that the Investigators' Report prepared for the Health Effects Institute (*9*), upon which this paper is based, accurately reflects the raw data and that deviations from the protocol have been considered and addressed appropriately in the analysis of the data and interpretation of the results of the study.

Results

Subjects

Sixty-three male subjects, with a mean age of 62 years (41–75 years), who had stable angina pectoris and a positive exercise test with ST-segment changes

Table 12. COHb levels in venous blood determined by gas chromatography and by CO oximetry.[a]

	Air day			2%-COHb-target day			4%-COHb-target day		
Method	*n*	%COHb	SEM[b]	*n*	%COHb	SEM	*n*	%COHb	SEM
After exercise test 1 (sample 2)									
GC	62	0.64	0.02	62	0.62	0.02	63	0.64	0.02
CO oximeter	63	1.24	0.05	62	1.24	0.05	63	1.23	0.04
After exposure (sample 5)									
GC target		—			2.2			4.4	
GC	59	0.70	0.02	56	2.38	0.05	59	4.66	0.09
CO oximeter	63	1.38	0.04	62	3.21	0.04	63	5.58	0.06
After exercise test 2 (sample 6)[c]									
GC target		—			2.0			4.0	
GC	63	0.62	0.02	62	2.00	0.05	63	3.87	0.08
CO oximeter	63	1.16	0.05	62	2.65	0.04	63	4.69	0.05

[a] Measurements were made on samples taken at the end of exercise test 1, at the end of the exposure period, and at the end of exercise test 2.
[b] SEM, standard error of the mean.
[c] Median values for %COHb in sample 6 are as follows: GC, air = 0.6, 2%-COHb target = 2.0, 4%-COHb target = 3.9; CO oximeter, air = 1.1, 2%-COHb target = 2.6, 4%-COHb target = 4.8.

Table 13. Individual center results for COHb levels in venous blood samples determined by gas chromatography.[a]

	Air day			2%-COHb-target day			4%-COHb-target day		
Center	*n*	%COHb	SEM[b]	*n*	%COHb	SEM	*n*	%COHb	SEM
After exercise test 1 (sample 2)									
Johns Hopkins	22	0.60	0.04	22	0.60	0.03	22	0.65	0.04
Rancho Los Amigos	17	0.77	0.03	18	0.69	0.05	18	0.72	0.05
St. Louis	23	0.58	0.04	22	0.57	0.04	23	0.57	0.04
After exposure (sample 5)									
Target		—			2.2			4.4	
Johns Hopkins	21	0.66	0.05	20	2.56	0.08	21	4.91	0.13
Rancho Los Amigos	15	0.78	0.04	14	2.59	0.06	15	4.99	0.10
St. Louis	23	0.68	0.04	22	2.09	0.08	23	4.22	0.17
After exercise test 2 (sample 6)[c]									
Target		—			2.0			4.0	
Johns Hopkins	22	0.58	0.05	22	2.25	0.06	22	4.00	0.12
Rancho Los Amigos	18	0.71	0.03	18	2.05	0.10	18	4.06	0.14
St. Louis	23	0.58	0.04	22	1.70	0.07	23	3.59	0.13

[a] Measurements were made in samples taken at the end of exercise test 1, at the end of the exposure period, and at the end of exercise test 2.
[b] SEM, standard error of the mean.
[c] Median values for %COHb in sample 6 are as follows: Johns Hopkins, air = 0.60, 2%-COHb target = 2.30, 4%-COHb target = 3.85; Rancho Los Amigos, air = 0.70, 2%-COHb target = 2.00, 4%-COHb target = 4.20; St. Louis, air = 0.60, 2%-COHb target = 1.70, 4 %-COHb target = 3.60.

on their ECGs suggestive of myocardial ischemia, completed all tests and met the protocol criteria (Tables 6 and 7). Each subject had at least one of three other specific indicators of coronary artery disease; 40 had angiographic evidence of at least a 70% narrowing in at least one major coronary artery, 26 had a documented prior myocardial infarction, and 27 had positive thallium stress tests. Additional information on these subjects and on the inclusion and exclusion criteria is provided in the methods section (Tables 6 and 7). Throughout the Results section, values are presented as means ± standard errors.

Carbon Monoxide Exposure

Exposure to CO in these subjects was varied individually to result in predetermined levels of COHb. Table 12 provides information on the mean COHb levels determined by GC and CO oximetry. The COHb levels measured by GC are more accurate; the CO oximetry COHb values are presented only for comparison with related studies. Table 12 shows that the combined mean COHb levels by GC at the end of the second exercise test were 2.00% ± 0.05% and 3.87% ± 0.08% for the 2%- and 4%-COHb target days, respectively. These values are close to the target levels of 2.0 and 4.0% at the end of exercise test 2, defined by the study protocol. The actual levels at the end of the CO exposure period, 2.38 and 4.66%, are slightly higher than the target levels of 2.2 and 4.4%. At all centers, the mean preexposure COHb levels were 0.6 to 0.7%. Table 13 shows that the mean COHb levels after CO exposures were higher at Johns Hopkins and Rancho Los Amigos than at St. Louis. The data in Table 13 show that there is an offset of 0.4 to 1.2% between GC and CO oximeter measurements.

The desired CO exposure concentrations were calculated on the basis of CO-uptake rate constants determined during visit 1 for each subject. The mean values and ranges for the CO chamber concentrations are shown in Table 14. The individual CO exposure concentrations on the 2%-COHb target day ranged from 42 to 202 ppm (mean of 117 ppm) and on the 4%-COHb target day from 143 to 357 ppm (mean of 253 ppm). The mean chamber concentrations on the 2%-COHb target day were 102 ppm at St. Louis, 116 ppm at Rancho Los Amigos, and 134 ppm at Johns Hopkins. On the 4%-COHb target day, the chamber concentrations were 237 ppm at St. Louis, 256 ppm at Rancho Los Amigos, and 267 ppm at Johns Hopkins. The COHb values at the end of exercise are lower than those at the end of exposure due to both respiratory loss of CO during the interval between the two measurements and the possibility of transfer of CO into extravascular compartments (*30,31*).

Table 14. Chamber carbon monoxide concentrations.[a]

	Air				2%-COHb target				4%-COHb target			
Center	*n*	Mean	SEM[b]	Range	*n*	Mean	SEM	Range	*n*	Mean	SEM	Range
Combined	62	0.7	0.1	0-2	62	117.4	4.4	42-202	62	252.9	6.1	143-357
Johns Hopkins	22	0.1	0.1	0-2	22	133.9	6.7	84-202	22	267.1	9.6	177-357
Rancho Los Amigos	18	1.3	0.1	1-2	18	115.7	8.3	42-170	18	255.8	12.7	143-315
St. Louis	22	0.8	0.1	0-2	22	102.3	6.5	56-174	22	237.0	9.3	150-315

[a] Concentrations are given in parts per million.
[b] SEM, standard error of the mean.

Table 15. Effect of carbon monoxide on time to ST end point (combined data).

		COHb levels at end of exercise pre- and postexposure			Time to ST end point pre- and postexposure, sec[a]		Change in time to ST end point post- vs. preexposure, sec		% Decrease between air and CO days			
Exposure day	Sample size		Mean %COHb[b]	SEM[c]		Trimmed mean	SEM	Trimmed mean	SEM	Trimmed mean, %[d]	*p*-Value[e]	90% Confidence interval	95% Confidence interval
Air	62	Pre	0.64	0.02	Pre	560.0	26.6	16.0	11.6				
		Post	0.62	0.02	Post	575.9	26.6						
2%-COHb target	61	Pre	0.62	0.02	Pre	574.1	26.8	−16.3	13.0	5.1	0.01	1.46, 8.74	0.77, 9.43
		Post	2.00	0.05	Post	557.8	25.4						
4%-COHb target	62	Pre	0.64	0.02	Pre	562.9	27.6	−52.7	12.7	12.1	≤0.0001	9.0, 15.3	8.4, 15.9
		Post	3.87	0.08	Post	510.1	25.9						

[a] Median time to ST end point; air, pre = 540, post = 570; 2%-COHb target, pre = 560, post = 524; 4%-COHb target, pre = 540, post = 500.
[b] CO measured by GC.
[c] SEM, standard error of the mean.
[d] Median percent decrease: air vs. 2%-COHb target = 6.5; air vs. 4%-COHb target = 13.2. For analysis of nontrimmed means, see Appendix C.
[e] One-sided *p*-values, as described in "Methods."

ST-End Point Analyses

The results of the ST-end point analyses are summarized in Table 15. For each subject, the percentage change between the pre- and postexposure exercise tests was calculated for each exposure day. Thus, each individual served as his own control on each day. The difference between the 2%-COHb day and air-exposure day was used for analysis of the effect of 2% COHb, and the difference between the 4%-COHb day and air-exposure day was used for the analysis of the effect of 4% COHb. A positive percentage indicates a decrease in time to ST end point due to CO exposure. It should be noted that the air exposure does not translate to zero COHb level, but rather gives the level of baseline COHb, resulting from endogenous CO production and CO exposure, which averages 0.6% in this sample.

As presented in Table 15, CO exposures that produced a mean level of 2% COHb resulted in a significant decrease in the time to ST end point ($p = 0.01$). Of the 61 subjects in the 2% analysis, 42 experienced a relative decrease and 19 experienced a relative increase on the 2%-COHb day, compared to the air day. On the air day, there was an average increase of 5.2% in time to ST end point on the postexposure exercise test relative to the preexposure test. In contrast, after the 2%-COHb exposure, the time to ST end point decreased an average of 0.3%. Thus, the 2%-COHb exposure resulted in a net 5.1% decrease (Table 15). (Note that because trimming is applied after all differences are calculated for each subject, the postexposure minus preexposure percentages reported for air-and 2%-COHb days do not sum to the mean decrease reported in Table 15.)

At the higher CO exposure level, a significant decrease in time to ST end point was also found ($p \leq 0.0001$). Of the 62 subjects in the 4% analyses, 49 experienced a relative decrease and 13 experienced a relative increase. Exposure to CO that produced a mean level of 3.9% COHb resulted in a 7.6% decrease in mean time to ST end point relative to the preexposure test. Thus, compared to the 5.2% postexposure increase in mean time to ST end point after the control air exposure, the 3.9%-COHb exposure resulted in a net 12.1%

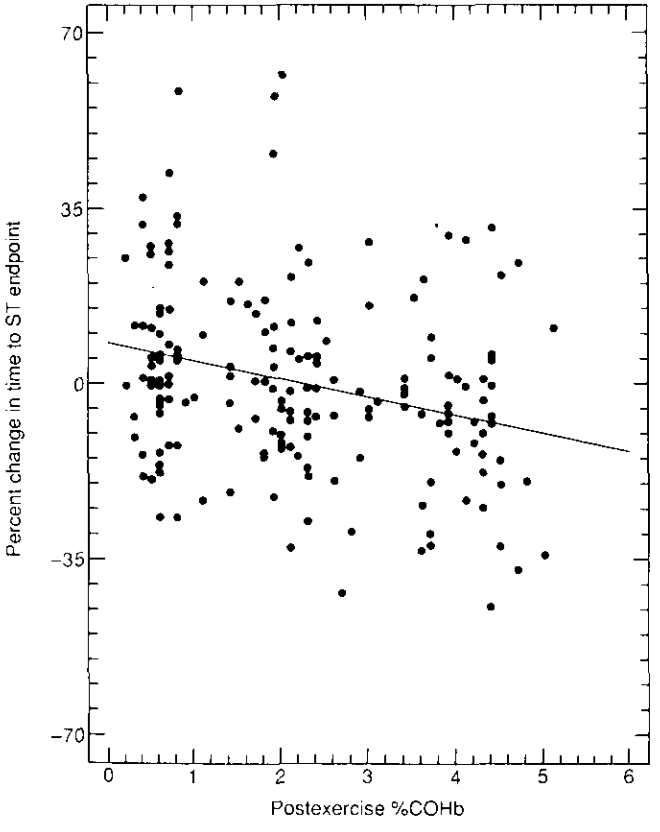

FIGURE 8. Regression of the percent change in time to ST end point between the pre- and postexposure exercise tests [(postexposure – preexposure)/preexposure] and the measured blood COHb levels at the end of exercise for the 63 subjects combined. The line represents the average of individual regressions.

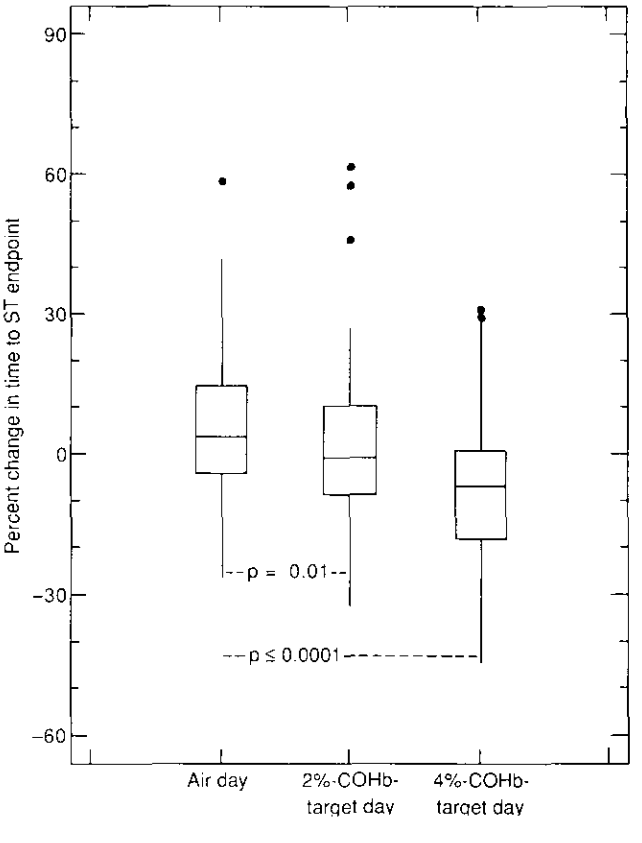

FIGURE 9. Box-and-whisker plots of percent change in time to ST end point between the pre- and postexposure exercise tests [(postexposure – preexposure)/preexposure] on the air day, 2%-COHb target day, and 4%-COHb target day. The bar across the box is the median, the ends of the box are the quartiles, the lines extend to the furthest points within 1.5 times the interquartile range from the box, and the separated dots represent individuals outside that range.

Table 16. Time to respective end points for subjects who had missing data.

Subject	Exposure	Time, sec					
		Exercise test 1 (preexposure)			Exercise test 2 (postexposure)		
		ST[a]	Angina[b]	Max[c]	ST	Angina	Max
101	4%-COHb target	—	1020	1039	960	885	1020
105	4%-COHb target	—	330	603	480	225	537
110	Air	900	902	990	—	665	1028
201	Air	—	605	630	—	727	755
	2%-COHb target	—	590	655	—	635	673
	4%-COHb target	540	365	660	—	380	600
214	2%-COHb target	—	452	697	810	499	986
215	4%-COHb target	—	1073	1113	920	1005	960

[a] ST, time to the onset of ST endpoint.
[b] Angina, time to onset of level 1 angina.
[c] Max, total duration of exercise.

Table 17. Effect of carbon monoxide on heart rate-systolic blood pressure double product at the onset of ST end point (combined data).

Exposure day	Sample size	Double product[a] at ST end point in pre- and postexposure exercise tests			Difference between pre- and postexposure exercise tests		% Decrease between air and CO days		
			Mean	SEM[b]	Mean	SEM	Mean %[c]	SEM	p-Value[d]
Air	61	Pre	16,134	563	214	320			
	61	Post	16,375	649					
2%-COHb target	60	Pre	16,558	620	−151	289	1.0	2.2	0.65
	61	Post	16,300	597					
4%-COHb target	58	Pre	15,986	575	−632	256	4.4	2.0	0.03
	62	Post	15,361	528					

[a] Beats per minute × mm Hg.
[b] SEM, standard error of the mean.
[c] Contrimmed means.
[d] Two-sided p-values.

decrease in time to ST end point (Table 15).

To assess a possible dose-response relationship, individual regressions were fitted to the three differences in time to ST end point, pre- versus postexposure, against the three actual COHb readings. When this was done, the average of the intercepts was 8.01% ± 2.48%, and the average of the slopes was −3.85% ± 0.63%/%COHb. This relationship is illustrated in Figure 8. Thus, in this range of COHb levels, there appears to be, roughly, a 3.9% decrease in time to ST end point for every increase of 1% in COHb. Figure 9 illustrates this dose-response relationship.

Six of the 63 subjects did not reach the ST end point in one or more of the 376 exercise tests (Table 16). One subject, 201, reached the ST end point in only one of the six exercise tests. Since the analyses are based on the differences between pre- and postexposure times, no information on ST end point is available on this subject, and thus he is not included in the ST analyses. One subject, 110, did not reach the ST end point on the postexposure test on the air day, and four subjects, 101, 105, 214, and 215, did not reach the ST end point on the preexposure exercise test on one of the days they were exposed to CO. In each of these instances, because we know that these subjects did not reach the ST end point by the end of exercise, the total duration of exercise was used instead of the time to ST end point in the analyses. The effect of these substitutions is minimal. If they produce any effect, their influence on study results and the statistical analyses will be to underestimate any changes due to CO.

The mean heart rate-systolic blood pressure double product (beats per minute × mm Hg) at the time of ST end point is presented in Table 17. On the air-exposure day, there was a mean 1.2% increase in the double product at the onset of ST end point on the postexposure test. On the 2%-COHb day, there was no significant change in the double product at the ST end point. However, on the 3.9%-COHb day, there was a 3.4% decrease in the double product on the postexposure exercise test, which represents a net 4.4% decrease ($p = 0.03$) compared to the air-exposure day. Thus, on the 3.9%-COHb day, the threshold ST change occurred both earlier in the exercise test and at a lower heart rate-blood pressure double product.

Table 18 presents data on the maximum ST amplitude during exercise in the selected ECG lead, and Table 19 presents data on the sum of the maximum changes in all leads with ST-segment changes equal to or greater than 0.5 mm. There was an 11% increase in the maximum amplitude of ST changes on the 2%-COHb day, compared to the air day ($p = 0.002$). On the

Table 18. Effect of carbon monoxide on maximum ST amplitude.

Exposure day	Sample size		Maximum ST on pre- and postexposure exercise tests, mm		Differences between pre- and postexposure exercise tests, mm		% Increase between air and CO days		
			Mean	SEM[a]	Mean	SEM	Mean %	SEM	p-Value[b]
Air	62	Pre	1.86	0.09					
	62	Post	1.66	0.09	−0.20	0.05			
2%-COHb target	61	Pre	1.85	0.11					
	61	Post	1.82	0.09	−0.02	0.05	11.4	3.5	0.002
4%-COHb target	61	Pre	1.85	0.10					
	62	Post	1.88	0.09	+0.03	0.05	17.2	4.1	≤ 0.0001

[a] SEM, standard error of the mean.
[b] Two-sided *p*-values.

Table 19. Effect of carbon monoxide on summed ST score.

Exposure day	Sample size		ST score[a] in pre- and postexposure exercise tests		Difference between pre- and postexposure exercise tests		% Increase between air and CO days		
			Mean	SEM[b]	Mean	SEM	Mean %	SEM	p-Value[c]
Air	63	Pre	7.4	0.4	−1.3	0.3			
	62	Post	6.2	0.4					
2%-COHb target	61	Pre	7.3	0.5					
	61	Post	7.0	0.4	−0.2	0.2	20.8	6.9	0.004
4%-COHb target	62	Pre	7.2	0.5					
	63	Post	7.0	0.4	−0.2	0.2	23.3	6.9	0.001

[a] ST score is the sum of the maximum changes in all leads with ST-segment changes ≥ 0.5 mm.
[b] SEM, standard error of the mean.
[c] Two-sided *p*-values.

Table 20. Effect of carbon monoxide on duration of ST segment changes.

Exposure day	Sample size		ST duration in pre- and postexposure exercise tests, sec		Difference between pre- and postexposure exercise tests, sec		% Increase between air and CO days		
			Mean	SEM[a]	Mean	SEM	Mean %	SEM	p-Value[b]
Air	61	Pre	118	20	21	12			
	61	Post	99	15					
2%-COHb target	61	Pre	118	19					
	60	Post	107	15	−10	12	61	61	0.32
4%-COHb target	58	Pre	130	22					
	62	Post	113	17	−12	11	− 7	32	0.84

[a] SEM, standard error of the mean.
[b] Two-sided *p*-values.

3.9% day, there was a 17% increase in the maximum ST amplitude, compared to the air day ($p \leq 0.0001$). The summed ST score showed a 21% increase on the 2%-COHb day ($p = 0.004$) and a 23% increase on the 3.9%-COHb day, compared to the air day ($p = 0.001$). The duration of ST-segment changes was measured (Table 20) but no changes in duration were found.

Angina Analyses

Figure 10 illustrates the significant correlation between change in time to ST end point and change in time to angina (Spearman rank correlation coefficient = 0.49, $p \leq 0.0001$; Pearson correlation coefficient = 0.49, $p \leq 0.0001$). The results for time to onset of angina are presented in Table 21 and in Figure 11. There was a significant decrease of 4.2% in time to onset of angina on the 2%-COHb day, compared to the air day ($p = 0.03$). Of the 62 subjects, 38 experienced a relative decrease in time to onset of angina and 23 a relative increase (one subject had no change). On the 3.9%-COHb day, there was also a significant decrease in time to

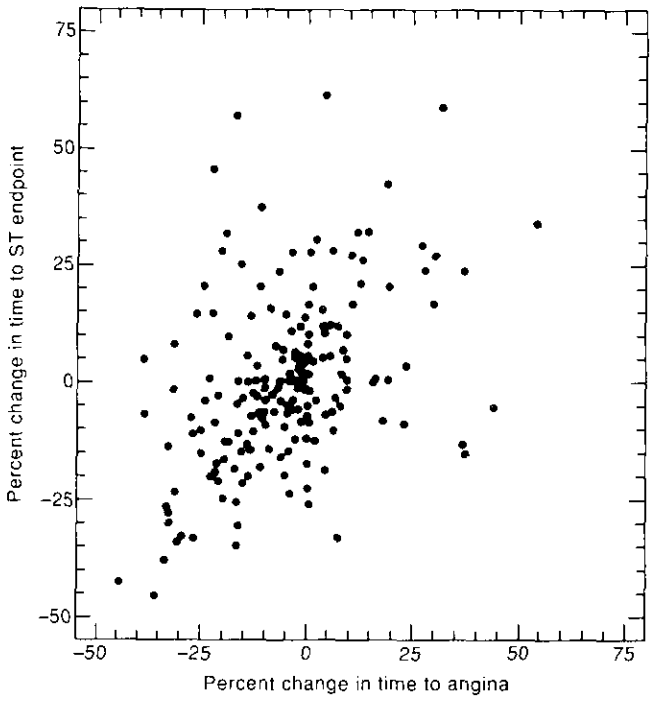

FIGURE 10. Relationship between percent change in time to ST end point [(postexposure – preexposure)/preexposure] and the percent change in time to angina [(postexposure – preexposure)/preexposure].

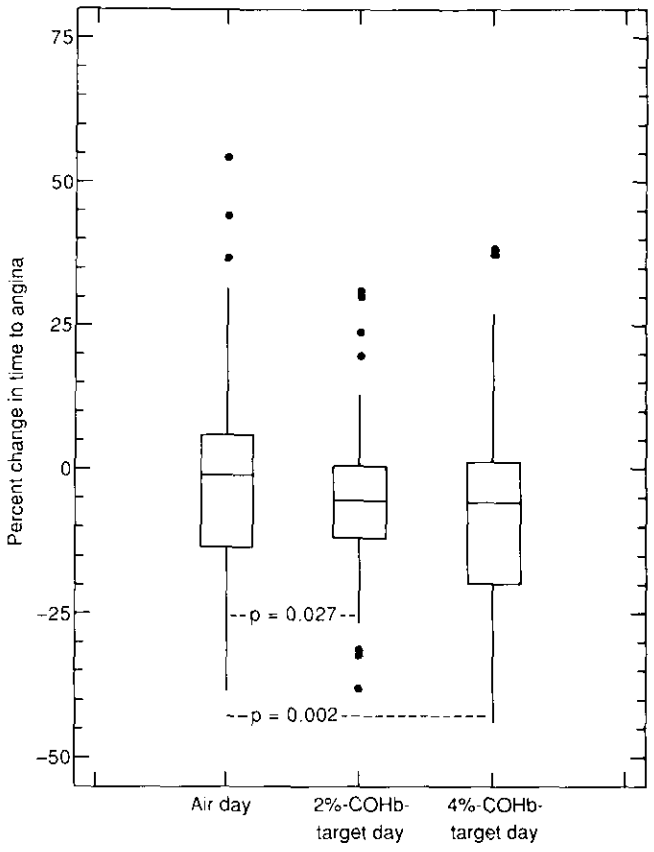

FIGURE 11. Box-and-whisker plots of percent change in time to angina between the pre- and postexposure exercise tests [(postexposure – preexposure)/preexposure] on the air day, the 2%-COHb target day, and the 4%-COHb target day. The bar across the box is the median, the ends of the box are the quartiles, the lines extend to the furthest points within 1.5 times the interquartile range from the box, and the separated dots represent individuals outside that range.

angina, with a mean decrease of 7.1% compared to the air day ($p = 0.002$). Of the 63 subjects, 45 experienced a relative decrease in time to onset of angina and 18 experienced a relative increase. There were no significant differences, however, between the air, 2%-COHb, and 3.9%-COHb days with respect to heart rate-blood pressure product at the onset of angina (Table 22).

Table 21. Effect of carbon monoxide on time to angina (combined data).

Exposure day	Sample size	COHb levels at end of exercise pre- and postexposure			Time to angina pre- and postexposure, sec[a]			Change in time to angina post- vs. preexposure, sec		% Decrease between air and CO days			
			Mean %COHb[b]	SEM[c]		Trimmed mean	SEM	Trimmed mean	SEM	Trimmed mean, %[d]	p-Value[e]	90% Confidence interval	95% Confidence interval
Air	63	Pre	0.64	0.02	Pre	519.0	26.7	−17.4	10.9				
		Post	0.62	0.02	Post	501.6	24.6						
2%-COHb target	62	Pre	0.62	0.02	Pre	525.2	26.2	−42.8	10.6	4.2	0.027	0.66, 7.94	0.4, 8.74
		Post	2.00	0.05	Post	482.4	22.0						
4%-COHb target	63	Pre	0.64	0.02	Pre	515.0	26.5	−49.6	10.9	7.1	0.002	3.06, 10.94	5.18, 14.46
		Post	3.87	0.08	Post	465.4	24.1						

[a] Median time to angina: air, pre = 520, post = 489; 2%-COHb target, pre = 482, post = 460; 4%-COHb target, pre = 480, post = 440.
[b] CO measured by GC.
[c] SEM, standard error of the mean.
[d] Median percent decrease; air vs. 2%-COHb target = 4.2; air vs. 4%-COHb target = 9.0. For analysis of nontrimmed means, see Appendix C.
[e] One sided p-values, as described in "Methods."

Table 22. Effect of carbon monoxide on heart rate-systolic blood pressure double product at the onset of angina (combined data).

Exposure day	Sample size		Double product[a] at angina in pre- and postexposure exercise tests		Difference between pre-and postexposure exercise tests		% Decrease between air and CO days		
			Mean	SEM[b]	Mean	SEM	Mean %[c]	SEM	*p*-Value[d]
Air	63	Pre	15,989	583					
	63	Post	15,225	572	−764	228			
2%-COHb target	61	Pre	15,822	596	−444	248	−2.0	1.8	0.26
	62	Post	15,365	571					
4%-COHb target	63	Pre	15,540	512	−562	190	−1.1	1.7	0.50
	63	Post	14,978	493					

[a] Beats per minute × mm Hg.
[b] SEM, standard error of the mean.
[c] Nontrimmed means.
[d] Two-sided *p*-values.

There was only one subject (306) with missing angina data; he did not experience angina on the preexposure exercise test on the 2%-COHb target day. The total duration of time on the treadmill was again used as the data point. The effect of estimating this value, as in the ST analyses, is minimal, and, if anything, makes the reported *p*-values conservative.

We also examined, as we did for the ST end point, the dose-response relationship between angina and COHb. The individual regressions resulted in an average intercept of −1% ± 2.11% and an average slope of −1.89% ± 0.81%/%COHb. The average decrease in time to angina pectoris appears to be roughly 1.9% for every 1% increase in COHb, over the range of COHb studied. This relationship is illustrated in Figure 12.

Total Exercise Duration

The effect of CO exposure on total exercise duration is presented in Table 23. On the air day, the mean total exercise duration was 692 ± 27 sec on the preexposure test and was 680 ± 26 sec on the postexposure test (a 1% decrease). On the 2%-COHb day, there was a 1.7% ± 1.6% decrease ($p = 0.29$) in the total exercise duration, compared to the air day. After exposure on the 3.9%-COHb day, there was a 6.2% decrease ($p \leq 0.0001$) in the total exercise duration, compared to the air day. There were no statistically significant differences between test days, however, with regard to the heart rate-systolic blood pressure double product at maximum exercise (Table 24).

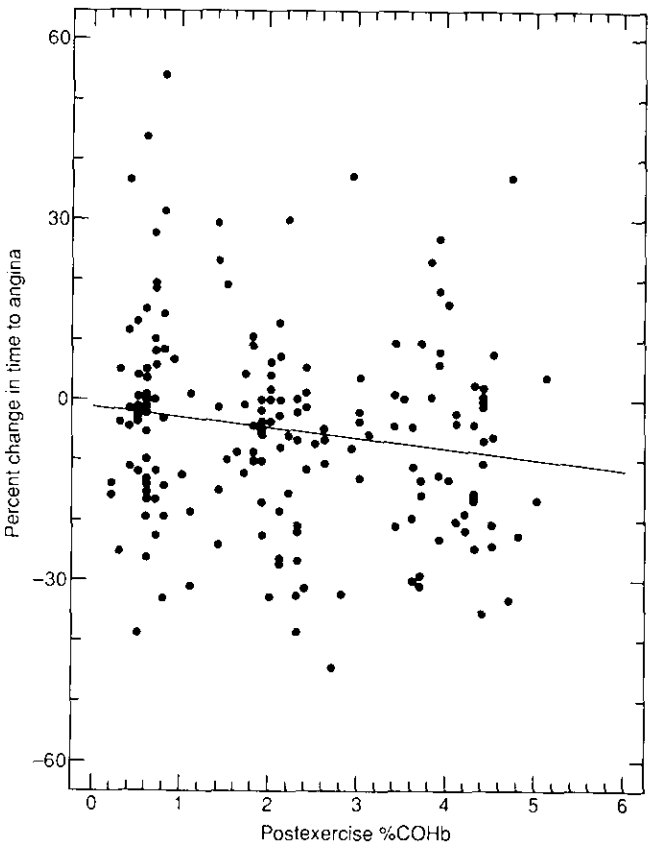

FIGURE 12. Regression of the percent change in time to angina between the pre- and postexposure exercise tests [(postexposure − preexposure)/preexposure] and the measured blood COHb levels at the end of exercise.

Covariate Analyses

The primary analyses evaluated the effect of COHb levels on the time to the ST and angina end points. Data were also collected on other factors that could influence these results. The factors generally fall into the categories of study design variables, anthropometric characteristics of the subjects, factors related to severity of an individual's coronary artery disease, factors that could affect cardiovascular performance or pulmonary function, variables related to the study centers, and the actual COHb levels. As described below, the actual COHb level was the most significant covariate.

The order in which the exposures were assigned to each subject was randomized to guard against a learning effect. The average times to reach ST end point for the preexposure exercise tests were 546 ± 8.3 sec at

Table 23. Effect of carbon monoxide on total exercise duration.

Exposure day	Sample size		Total exercise time in pre- and postexposure tests, sec		Change in time, sec		% Decrease between air and CO days	
			Mean	SEM[a]	Mean	SEM	Mean %	p-Value[b]
Air	63	Pre	692	27	-12.1	8.2	—	—
		Post	680	26				
2%-COHb target	62	Pre	700	26	-27.0	9.7	1.7	0.29
		Post	673	24				
4%-COHb target	63	Pre	693	27	-53.0	9.0	6.2	≤ 0.0001
		Post	640	26				

[a] SEM, standard error of the mean.
[b] Two-sided p-values.

Table 24. Effect of carbon monoxide on heart rate-systolic blood pressure double product at peak exercise.

Exposure day	Sample size		Double product[a] at peak Exercise in pre- and postexposure tests		Difference in double product (pre- minus postexposure mean difference)		% Decrease between air and CO days	
			Mean	SEM[b]	Mean	SEM	Mean %	p-Value[c]
Air	63	Pre	18,486	649	-285	265	—	—
	63	Post	18,201	748				
2%-COHb target	62	Pre	18,700	718	61	232	-3.23	0.08
	62	Post	18,761	678				
4%-COHb target	63	Pre	18,761	637	-569	243	0.23	0.89
	63	Post	17,686	590				

[a] Beats per minute × mm Hg.
[b] SEM, standard error of the mean.
[c] Two-sided p-values.

visit 2, 569 ± 8.5 sec at visit 3, and 580 ± 8.4 sec at visit 4. The learning trend is apparent (p = 0.02). For the time to angina, the three averages were: 498 ± 9.1 sec, 528 ± 9.2 and 532 ± 9.1 sec (p = 0.02). For the analyses that examined the effect of CO, the raw values were not used, but rather the difference between the pre- and postexposure values were used. The question, then, is whether the order in which the exposures were assigned had an effect on these differences. To answer this question, analyses of covariance were performed on both the time to ST end point and the time to angina for the differences in seconds. In these analyses the order in which the exposures were assigned, together with the actual COHb percentage, was tested. In none of the analyses was the order of exposures significant.

Regressions were performed to determine whether or not the differences found in both the ST end point and angina analyses could be explained by other measured covariates. Four sets (decrease in percentages in time to ST end point and time to angina for both the 2%-COHb target data and the 4%-COHb target data) of simple regressions were performed (each involving one covariate). In each case, to guard against possible effects of nonlinearity and outliers, we performed a rank regression with normal scores for all variables. In each set of regressions, the individual covariates were the subject's age, smoking history, history of hypertension, prior myocardial infarction, occupation, use of beta-blockers, use of other medications, height, weight, the maximal time spent on the treadmill at visit 1, the average time to angina on three preexposure exercise tests, QRS duration, sodium level, potassium level, chloride level, FEV_1, percent of predicted FEV_1, FVC, percent of predicted FVC, the study center, and the actual COHb level by GC. The actual COHb level was significant for the analyses of time to ST end point (for 2%-COHb target analysis, p = 0.014; for 4%-COHb target analysis, p = 0.014), but was not significant for the angina analyses (for 2%- COHb target analysis, p = 0.86; for 4%-COHb target analysis, p = 0.87). The only other significant ($p \leq 0.05$) covariate was weight for the time to angina analysis at the 4%-COHb level. With 84 (4 × 21) regression coefficients, we would expect an average of about four to be significant at 5% by pure chance, compared to the three that were found.

The fact that the time spent on the treadmill on the first exercise test of visit 1 was not a significant covariate indicates that the percent change due to CO is not correlated with the severity of limitation of exercise capacity. This conclusion is also supported by the fact that the average time to angina in the preexposure exercise tests of visits 2, 3, and 4 was not a significant

Table 25. Effect of carbon monoxide on time to ST end point at the three centers.

Exposure day	Sample size		COHb levels at end of exercise pre- and postexposure: Mean COHb[b]	SEM[c]		Time to ST end point pre- and postexposure, sec[a]: Mean	SEM	Change in time to ST end point post- vs. preexposure, sec: Mean	SEM	% Decrease between air and CO days: Trimmed mean, %[d]	p-Value[e]	90% Confidence interval	95% Confidence interval
Johns Hopkins University													
Air	22	Pre	0.60	0.04	Pre	596.0	48.9	7.1	21.8				
		Post	0.58	0.05	Post	603.1	50.7						
2%-COHb target	22	Pre	0.60	0.03	Pre	601.8	47.7	-29.6	29.3	5.9	0.042	0.27, 11.73	- 0.1, 12.84
		Post	2.25	0.06	Post	572.2	47.7						
4%-COHb target	22	Pre	0.65	0.04	Pre	610.1	48.0	-88.7	25.7	17.5	≤ 0.0001	11.67, 22.02	10.36, 22.93
		Post	4.00	0.12	Post	521.4	46.7						
Rancho Los Amigos Medical Center													
Air	17	Pre	0.77	0.03	Pre	580.0	45.7	51.8	24.8				
		Post	0.71	0.03	Post	631.8	42.8						
2%-COHb target	17	Pre	0.69	0.05	Pre	606.8	39.6	- 5.4	17.3	10.8	0.0003	6.08, 17.24	5.18, 18.91
		Post	2.05	0.10	Post	601.2	40.7						
4%-COHb target	17	Pre	0.72	0.05	Pre	588.4	54.8	-20.8	26.7	10.5	0.008	3.78, 17.84	2.29, 19.62
		Post	4.06	0.14	Post	567.6	49.1						
St. Louis University													
Air	23	Pre	0.58	0.04	Pre	510.6	42.3	- 2.0	13.8				
		Post	0.58	0.04	Post	508.6	40.0						
2%-COHb target	22	Pre	0.57	0.04	Pre	521.2	48.0	-11.3	16.8	-1.1	> 0.5	- 8.44, 5.33	-10.03, 6.53
		Post	1.70	0.07	Post	509.9	41.1						
4%-COHb target	23	Pre	0.57	0.04	Pre	498.8	41.1	-41.9	11.0	9.0	0.025	4.35, 13.64	3.47, 14.63
		Post	3.59	0.13	Post	456.9	38.6						

[a] Median time to ST end point; Johns Hopkins, air, pre = 570.0, post = 555.0; 2%-COHb target, pre = 570.0, post = 522.0; 4%-COHb target, pre = 611.5, post = 480.0. Rancho Los Amigos, air, pre = 600.0, post = 630.0; 2%-COHb target, pre = 600.0, post = 590.0; 4%-COHb target, pre = 550.0 post = 540.0. St. Louis, air, pre = 480.0, post = 480.0; 2%-COHb target, pre = 532.5, post = 480.0; 4%-COHb target, pre = 490.0, post = 450.0.

[b] CO measured by GC.

[c] SEM, standard error of the mean.

[d] Median percent decrease; Johns Hopkins, air vs. 2%-COHb target = 7.7; air vs. 4%-COHb target = 20.3, Rancho Los Amigos, air vs. 2%-COHb target = 8.5; air vs. 4%-COHb target = 12.7. St. Louis, air vs. 2%-COHb target = 1.0; air vs. 4%-COHb target - 7.6. For analysis of nontrimmed means, see Appendix A.

[e] One-sided *p*-values, as described in "Methods."

covariate. Thus, subjects with varying degrees of cardiac disease have similar percent effects of CO on their exercise capacity.

Individual Center Analyses

Table 25 presents data on ST end point by center. The 2%-COHb-target exposure level, compared to the air day, produced a statistically significant effect on time to ST end point at Johns Hopkins and Rancho Los Amigos, but not at St. Louis. The 4%- COHb-target exposure level produced a statistically significant decrease in time to ST end point at all three centers, with a range of 9.0 to 17.5%. Table 25 shows that the effect on ST end point was greater after the 4%-COHb target exposure than after the 2%-COHb target exposure at two centers, Johns Hopkins and St. Louis, but not at Rancho Los Amigos.

The box-and-whisker plots shown in Figure 13 depict this analysis. These plots show the overlap among the centers and incorporate the differing average COHb levels at the different centers, thus the slight increase in reading from left to right. Analysis of covariance was first performed with the raw differences in seconds as responses and the measured COHb levels as covariates. Next, center indicator variables were introduced as individual covariates. This analysis was repeated for the percent difference as responses. In neither analysis did the center prove significant; only the COHb level was significant.

Table 26 presents results of the angina analyses at the three centers. The effect of the 2%-COHb target exposure is statistically significant at two centers, Johns Hopkins and Rancho Los Amigos. The 4%-COHb tar-

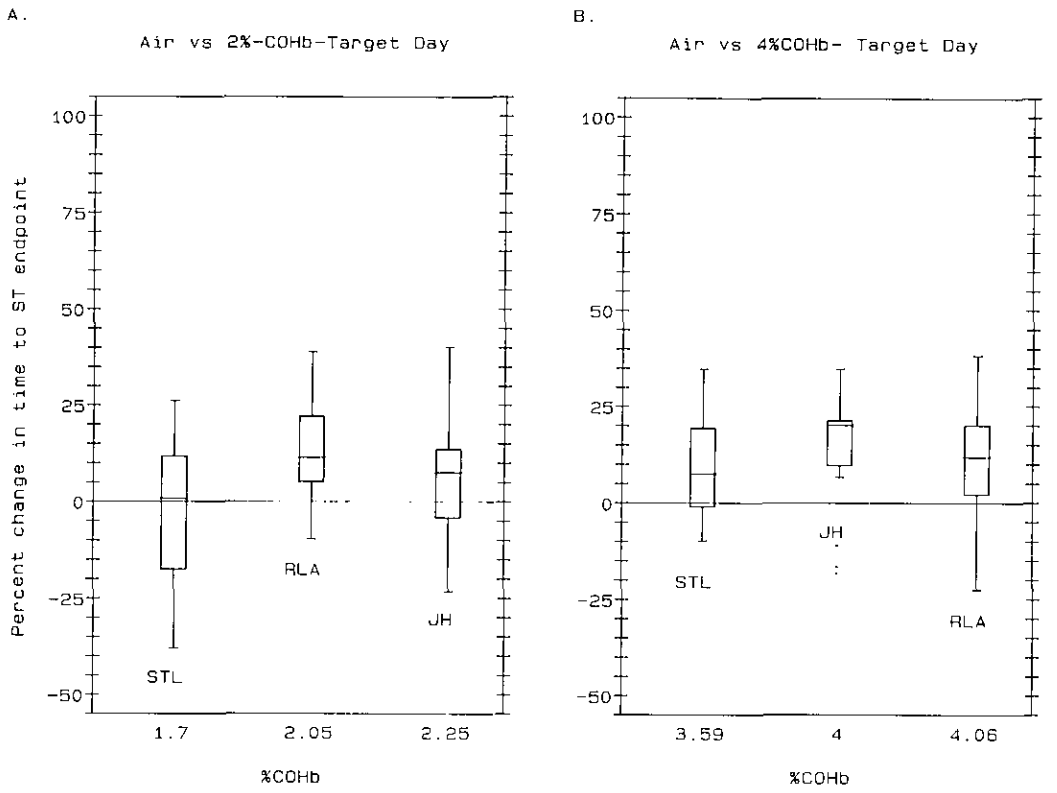

FIGURE 13. Box-and-whisker plots of individual differences in percentages of time to ST end point on air day compared to CO days at the three centers, showing the mean COHb level at the end of the exercise test after CO exposure at each center. The bar across the box is the median, the ends of the box are quartiles, the lines extend to the furthest points within 1.5 times the interquartile range from the box, and the separated dots represent individuals beyond that range. The dashed line is at a difference of 0. (*A*) Results for the air versus 2%-COHb target day, (*B*) results for the air versus 4%-COHb target day.

get exposure also had a significant effect at only two centers, Rancho Los Amigos and St. Louis. The box-and-whisker plots shown in Figure 14 depict these analyses. As with the ST analyses, these plots show the overlaps among the centers and incorporate the differing average COHb levels at the different centers. As with the ST end point, analysis of covariance was performed using the raw differences, and then the percent differences, as responses. In neither analysis did the center prove significant; again, only the COHb level was significant.

Discussion

Biological Effects

This study demonstrates, with the use of objective ECG measurements, the influence of low doses of CO (2% COHb) on the development of ischemia in subjects with coronary artery disease. Carbon monoxide exposure caused a reduction in the time to ischemic ST-segment changes during exercise (Table 15). The earlier attainment of this level of ischemia and the increase in the maximal change in ST segments (Table 18) and summed ST segment changes (Table 19) during exercise indicate that low doses of CO limit exercise tolerance in this group of individuals. This limitation is further demonstrated at the 3.9%-COHb level, at which the duration of the symptom-limited exercise test was reduced. The subjective end point of time to onset of angina was also shortened at both the 2.0%- and 3.9%-COHb levels. As expected, the ECG and angina end points are closely correlated ($R = 0.49$, $p \leq 0.0001$) (Fig. 10) and together strongly suggest that these low doses of CO produce a decrease in oxygen availability to the myocardium in subjects with coronary-blood-flow limitation. Furthermore, there is a significant dose-response relationship between COHb levels and the decrease in time to ST end point (Fig. 8). There was a 3.9% decrease in time to ST end point for every 1% increase in COHb. All of these experimental results indicate that low doses of CO exposure affect cardiac function during exercise by facilitating the development of ischemia. The most likely mechanism responsible for these changes is a reduction in the oxygen-carrying capacity of the blood, but more complex effects of CO on myocardial function cannot be excluded.

Starling and co-workers performed experiments that were similar in design to this protocol: subjects with coronary artery disease exercised twice each day on 2

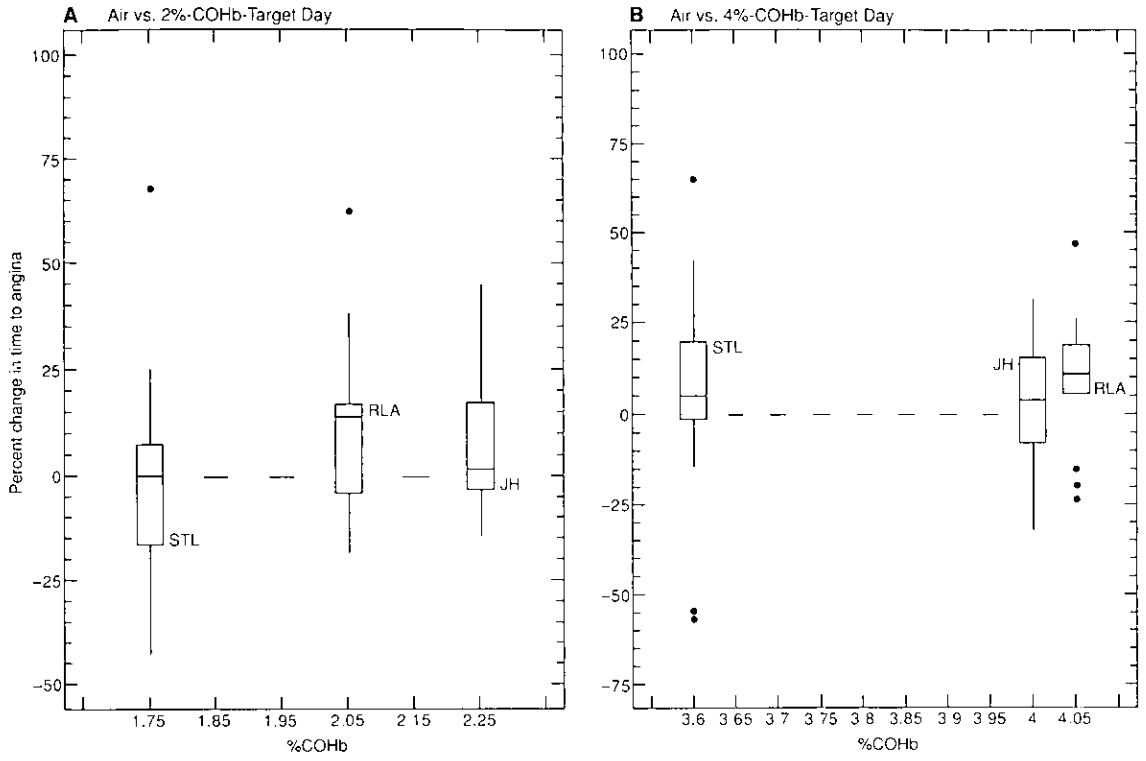

FIGURE 14. Box-and-whisker plots of individual differences in percentages of time to angina on air compared to CO days at the three centers, showing the mean COHb level at the end of the exercise test after CO exposure at each center. The bar across the box is the median, the ends of the box are quartiles, the lines extend to the furthest points within 1.5 times the interquartile range from the box, and the separated dots represent individuals beyond that range. The dashed line is at a difference of 0. (*A*) Results for the air versus 2%-COHb target day; (*B*) results for the air versus 4%-COHb target day.

days separated by less than 1 week. The study design permitted analysis of the results of repeated exercise tests between days and between tests on the same day. Virtually all of the cardiovascular and exercise parameters reported by Starling had reduced variability when measured on the same day, relative to tests performed on different days. Starling and co-workers reported that the time to onset of angina and time to 0.1-mV ST-segment change was increased in the second test on the same day, although the heart rate-blood pressure double product was unchanged at these time points in the exercise test (*11*). The results of Starling and associates are similar to the increase in time to ST end point observed in the present study on exercise testing after air exposure.

Repeated exercise testing on the same day has been used for a variety of purposes. Wayne and LaPlace (*32*) used the method to test the efficacy of atropine in patients with exercise-induced angina pectoris. The study of the acute effects of pharmacologic agents has been the major use of repeated exercise testing (*33,34*). There has been disagreement regarding the effects of the initial bout of exercise on the subsequent exercise tests performed on the same day. A warm-up phenomenon (improvement in performance on the second test) has been shown by several investigators (*11,34–36*). However, other studies have reported no change in performance on subsequent exercise tests (*7,32,33,37,38*). These differences in results do not appear to be due to the interval between tests nor to the type of test.

In this study, the use of repeated exercise tests was based on the report of Starling et al. (*11*). These investigators demonstrated that the variability in the end points of interest was reduced significantly between two tests performed on the same day relative to tests on separate days. The variability in measurement of angina responses during exercise is well known (*11*). Because of the demonstrated use of testing twice on the same day without significant health risks to the subjects, two tests were used to reduce the variability and thereby increase the power of the study design. The potential for the warm-up phenomenon was addressed by the use of a placebo (air) day in the study design. As in drug testing studies using multiple exercise tests on the same day, the CO effects were all determined relative to the placebo (air day) test results.

The physiological significance of the use of the time to 1-mm ST segment change in subjects with stable angina has been demonstrated by Starling et al. (*11*) and by Waters et al. (*36*). These studies reported a high correlation of the product of heart rate and blood pressure with the time to 1-mm ST segment change on mul-

Table 26. Effect of carbon monoxide on time to angina at the three centers.

Exposure day	Sample size	COHb levels at End of exercise pre- and postexposure: Mean %COHb[b]	SEM[c]	Time to angina pre- and postexposure, sec[a]	Mean	SEM	Change in time to angina post- vs. preexposure, sec: Mean	SEM	% Decrease between air and CO days: Trimmed mean, %[d]	p-Value	90% Confidence interval	95% Confidence interval
Johns Hopkins University												
Air	22	Pre	0.60	0.04	Pre	562.2	57.5	−46.1	16.9			
		Post	0.58	0.05	Post	516.0	51.2					
2%-COHb target	22	Pre	0.60	0.03	Pre	547.7	53.9	−76.2	19.6	5.2	0.014	0.48, 9.93	− 0.42, 11.03
		Post	2.25	0.06	Post	471.4	46.1						
4%-COHb target	22	Pre	0.65	0.04	Pre	557.1	51.7	−62.2	18.9	4.6	0.09	−0.32, 10.44	− 2.53, 11.53
		Post	4.00	0.12	Post	494.9	49.4						
Rancho Los Amigos Medical Center													
Air	18	Pre	0.77	0.03	Pre	541.5	43.0	14.7	22.7				
		Post	0.71	0.03	Post	556.2	41.7						
2%-COHb target	18	Pre	0.69	0.05	Pre	555.9	40.6	−35.7	15.7	9.4	0.01	3.18, 16.82	1.78, 18.21
		Post	2.05	0.10	Post	520.2	36.3						
4%-COHb target	18	Pre	0.72	0.05	Pre	522.9	48.7	−33.0	21.1	10.4	0.002	2.18, 17.44	0.48, 18.73
		Post	4.06	0.14	Post	489.9	47.6						
St. Louis University													
Air	23	Pre	0.58	0.04	Pre	460.0	33.3	−15.0	16.4				
		Post	0.58	0.04	Post	445.1	31.4						
2%-COHb target	22	Pre	0.57	0.04	Pre	477.6	37.9	−15.2	16.6	− 3.2	> 0.5	−9.44, 3.44	−10.83, 4.93
		Post	1.70	0.07	Post	462.4	29.5						
4%-COHb target	23	Pre	0.57	0.04	Pre	468.4	37.0	−50.5	17.4	7.7	0.045	0.28, 14.23	− 1.51, 15.54
		Post	3.59	0.13	Post	418.0	26.8						

[a] Median time to angina: Johns Hopkins, air, pre = 535.0, Post = 492.5; 2%-COHb target, pre = 512.5, post = 430.0; 4%-COHb target, pre = 562.0, post = 452.5, Rancho Los Amigos, air, pre = 545.5, post = 478.5; 2%-COHb target, pre = 500.0, post = 491.5; 4%-COHb target, pre = 466.0, post = 439.0, St. Louis, air, pre = 450.0, post = 480.0; 2%-COHb target, pre = 448.5, post = 452.5; 4%-COHb target, pre = 470.0, post = 435.0.

[b] CO measured by GC.

[c] SEM, standard error of the mean.

[d] Median percent decrease; Johns Hopkins, air vs. 2%-COHb target = 2.5; air vs. 4%-COHb target = 4.3, Rancho Los Amigos, air vs. 2%-COHb target = 13.4; air vs. 4%-COHb target = 13.1, St. Louis, air vs. 2%-COHb target = 0.1; air vs. 4%-COHb target = 5.3. For analysis of nontrimmed means, see Appendix A.

[e] One-sided *p*-values, as described in the Methods section.

tiple exercise tests. Subjects with coronary artery disease who undergo repeated exercise tests show some variability both in the amount of work that they can perform and in the time required to reach significant ST segment changes. However, it has been demonstrated that 1-mm ST segment change occurs at a relatively constant double product regardless of the time course of the exercise required to reach the level of double product necessary for significant ST change. Since the double product is a reflection of the myocardial work, the use of time to 1-mm ST change represents a measurement of myocardial ischemia at a work load independent of training or warm-up effects.

The reduction in double product required to reach a 1-mm ST segment change following 3.9% COHb indicates that significant ischemia had occurred at a lower level of myocardial work. The limited significance in the changes in double product observed in these studies reflects to some extent the fact that the measurements of blood pressure were carried out every minute. If continuous measurements of blood pressure had been made, it might have been possible to see the expected dose-response of double product relative to the dose-response in the time to ST with increased levels of COHb.

The heart rate-systolic blood pressure double product provides a clinical index of the work of the heart and myocardial oxygen consumption (*39*), since heart rate and blood pressure are two of the major determinants of myocardial oxygen consumption (*40*). If low levels of COHb significantly reduce oxygen delivery to the myocardium, then the development of ischemia at a lower double product (or lower level of myocardial oxygen consumption) should be expected. The results for the heart rate-systolic blood pressure double product at the onset of ST end point show this trend (Table 17). On the

3.9%-COHb day, there was a statistically significant reduction in double product on the postexposure test. On the 2%-COHb day, there was also a decrease in the double product, but this change was not statistically significant. The earlier onset of ischemic ST segment changes at a lower double product (level of myocardial oxygen consumption) is consistent with the expected inability of these subjects to increase blood flow to meet myocardial oxygen demands. In these subjects, when coronary blood flow reaches its limit, the reduction in arterial oxygen content by CO results in earlier development of ischemia.

Electrocardiographic evidence of ischemia occurred at lower levels of exercise with CO exposure than with ambient air exposure. It is implied that at a constant level of treadmill exercise, and therefore a constant myocardial workload, exposure to CO would result in greater myocardial ischemia (larger decreases in ST segment). This concept is partially supported by the analysis of end-of-exercise data. For example, in the 3.9%-COHb experiments, there was a significantly greater maximal change in ST segments observed at the end of exercise (Table 18). This occurred at a lower workload (Table 23) and at a significantly lower double product (Table 17). These findings further demonstrate augmentation of myocardial ischemia by increases in %COHb.

The primary mechanism responsible for the ability of CO to decrease oxygen delivery to the myocardium is the direct reduction in the oxygen-carrying capacity of the blood. This occurs even at very low partial pressures of CO because of the high affinity of hemoglobin for CO relative to oxygen (*41*). Several other factors may influence myocardial oxygenation and function. The combination of CO with hemoglobin can influence the available binding sites for oxygen, increasing the overall affinity of hemoglobin for oxygen (that is, a leftward shift in the oxygen-dissociation curve). The magnitude of this shift can be calculated from the change in pO_2 required to half-saturate available hemoglobin. In humans, this value is approximately 0.35 torr O_2/1% COHb (*10*). In the present study, this would mean 0.5-torr and 1.1-torr shifts in the oxygen-dissociation curve for the 2.0%- and 3.9%-COHb levels, respectively. In addition to reduced oxygen delivery, there is the potential for reduced tissue uptake of oxygen in the presence of CO. The uptake of oxygen in cardiac muscle is dependent upon the availability of myoglobin and the ability to transfer oxygen from myoglobin to cytochrome oxidase. However, it is less likely that cytochrome oxidase is affected by the low levels of COHb achieved in this study, since Chance and co-workers (*42*) have reported a negligible effect of CO on this part of the respiratory chain. The additional contributions by these other factors on oxygen availability and cardiac function are unknown and require further investigation.

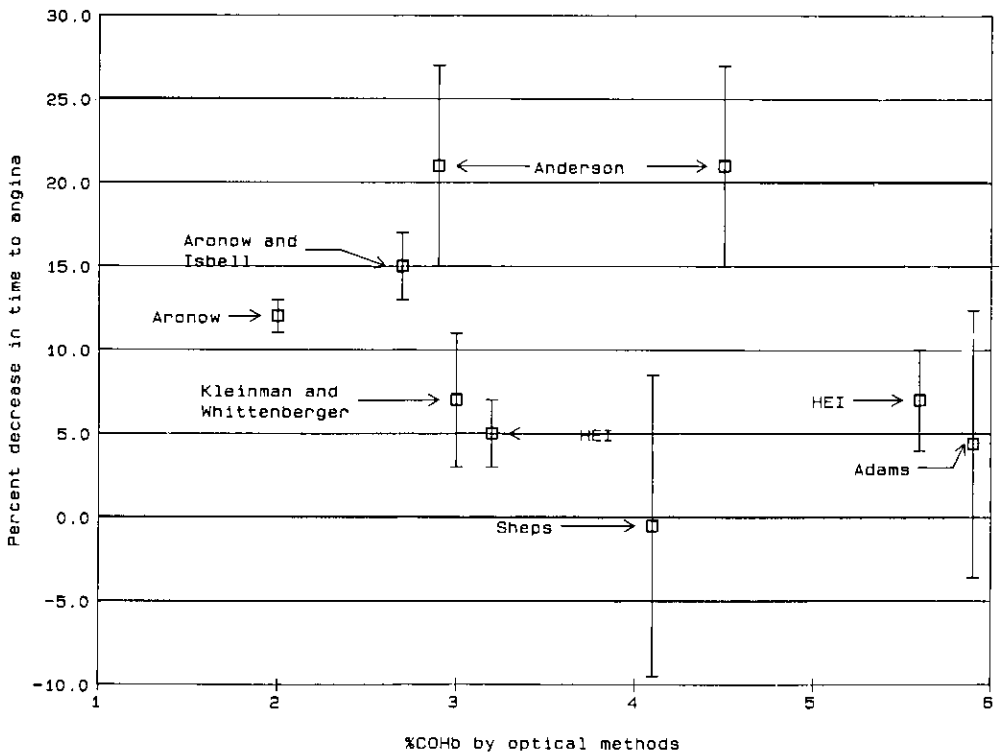

FIGURE 15. Comparison of effect of CO exposure on time to angina in several studies (see Tables 27 and 28 for more detailed information). The regression line is based on data from this study.

Table 27. Comparison of studies evaluating the effect of carbon monoxide on the time to onset of angina in subjects with coronary artery disease.

Investigators	No. of subjects	Exposures	%COHb, mean ± SD[a]		Effect of CO on time to angina[b]	
			Spectrophotometry	Gas chromatography	% Decrease CO day vs. air day	Standard error of mean
Aronow and Isbell (*5*)	10	Air/2 hr[c]	0.8 ± 0.2[d]	—	—	
		50 ppm CO/2 hr[c]	2.7 ± 0.2[d]	—	15	2
Aronow (*7*)	15	Air/1 hr[c]	1.0 ± 0.1[e]	—	—	
		50 ppm CO/1 hr[c]	2.0 ± 0.2[e]	—	12	1
Anderson et al. (*6*)	10	Air/4 hr[c]	1.3 ± 0.4[f]	—	—	
		50 ppm CO/4 hr[c]	2.9 ± 0.7[f]	—	21	6
		100 ppm CO/4 hr[c]	4.5 ± 0.8[f]	—	21	6
Kleinman (*19,43*)	26	Air/1 hr[c]	1.4 ± 0.5[e] (1.4 ± 0.5)[e]	—	—	
		100 ppm CO/1 hr[c]	3.0 ± 0.5[e] (2.8 ± 0.5)[e]	—	7	4
Sheps et al. (*17*)	23[g]	Air/about 1 hr[h]	1.7[e]	—	—	
		100 ppm CO/hr[h] about 1 hr	4.1[e] (3.6)[e]	—	0[i]	9
Adams et al. (*18*)	21[j]	Air/about 1 hr[h]	1.6[e] (1.6)[e]	—	—	
		100 or 200 ppm CO[h] about 1 hr	5.9[e] (5.2)[e]	—	4[i]	8
Allred at al. (*8,9*)	63	0–2 ppm CO mean 0.69/50–70 min	1.4 ± 0.4[e] (1.2 ± 0.4)[e]	0.70 ± 0.19 (0.62 ± 0.19)	—	
		42–202 ppm CO[h] mean 117/50–70 min	3.2 ± 0.3[e] (2.6 ± 0.3)[e]	2.38 ± 0.40 (2.00 ± 0.41)	5	2
		143–357 ppm CO[h] mean 253/50–70 min	5.6 ± 0.5[e] (4.7 ± 0.4)[e]	4.66 ± 0.62 (3.87 ± 0.62)	7	3

[a] %COHb at end of exposure. We calculated SD for Kleinman and Whittenberger (*19,43*) data. Data were not available to calculate SD for Sheps (*17*) and Adams (*18*) papers. Numbers in parentheses are %COHb at end of the exercise test after exposure period.

[b] The design of our study (*8,9*) was the same as the two Aronow studies (*5,7*) in that it had a pre- and postexposure exercise test each day. For those studies, we calculated the pairwise percent change between the tests on each day and then, pairwise, subtracted the result on the CO day from the result on the air day. For the Kleinman (*43*), Sheps (*17*), and Adams (*18*) data, we calculated the pairwise percent change between the air and CO days. For the Anderson (*6*) data, we calculated the pairwise percent changes between the randomized air day and the CO days (as did Anderson). We used the average of the two nonrandomized air days for the two subjects for whom data from the randomized air day were not available. For comparison with the other studies, the percentages listed in this table for our study are means (see Appendix B) rather than trimmed means (Table 21). We calculated SEM for all studies.

[c] Mask exposure.

[d] IL 182 CO oximeter.

[e] IL 282 CO oximeter.

[f] Spectrophotometric method.

[g] Thirty subjects in study, but angina analyzed in only 19 of them.

[h] Chamber exposure.

[i] Not statistically significant by one-tailed t-test.

[j] Thirty subjects in study, but angina analyzed in only 21 of them.

Comparison with Other Studies

This study was designed to assess the effects of low doses of CO on exercise function in subjects with coronary artery disease. The following parameters were incorporated into the protocol: *a)* a large well-defined study population, drawn from three geographical areas, with unequivocal evidence of ischemic cardiovascular disease; *b)* accurate assessment of CO exposure and COHb levels; *c)* exercise testing appropriate for the subject population; *d)* objective cardiovascular end points to assess the effects of CO exposure; *e)* a clearly defined protocol for subject testing and the main data analysis; and *f)* quality assurance procedures to assure that the protocol and standard operating procedures were followed and that data were reliable and traceable. Several protocol procedures were adopted to reduce variability in the data to increase

Table 28. Comparison of subjects in studies of the effect of carbon monoxide exposure on occurrence of angina during exercise.

Study	No. of subjects	Subject characteristics				
		Gender	Medication	Smoking history	Description of disease	Age, years
Aronow and Isbell (*5*)	10	Male	Not described	No current smokers	Classic exertional angina, CAD with > 50% stenosis of 1 or more major vessels	40–55 (mean = 49)
Aronow (*7*)	15	14 Male 1 Female	Not described	No current smokers	Stable angina pectoris with angiographically demonstrated CAD; 8 had prior MI	50.0 ± 7.2
Anderson et al. (*6*)	10	Male	1 subject took digitalis; drug therapy basis for exclusion	5 smokers (refrained for 12 hr prior to exposure)	Stable angina pectoris, positive exercise test (ST changes); reproducible angina on treadmill	Mean = 49.9
Kleinman and Whittenberger (*19,43*)	26	Male	14 on beta-blockers, 19 on nitrates	No current smokers	Ischemic heart disease, stable exertional angina pectoris	49–66 (Mean = 59)
Sheps et al. (*17*)	30 (19 with angina)	25 Male 5 Female	26 subjects on medication; 19 on beta-blockers; 11 on Ca-channel blockers; 1 long-acting nitrates	No current smokers	Ischemia during exercise (ST changes or abnormal ejection fraction response) and 1 or more of angiographically proven CAD; prior MI; typical angina	36–75 (Mean = 58.2)
Adams (*18*)	30 (21 with angina)	22 Male 8 Female	25 subjects on medication; 19 on beta-blockers; 13 on Ca-channel blockers; 1 on long-acting nitrates	No current smokers	One or more of ≥ 70% lesion by angiography in 1 or more major vessels; prior MI; typical angina or positive exercise test (ST changes) or both	58 ± 11
Allred et al. (*8,9*)	63	Male	38 subjects on beta-blockers; 36 on nitrates; 40 on Ca-channel blockers	No current smokers	Stable exertional angina and positive exercise test (ST changes) plus one or more of ≥ 70% lesion by angiography in 1 or more major vessels; prior MI; positive thallium stress test	41–75 (mean = 62.1)

the statistical power of the analyses that were to be used. The three major procedures involved an exposure protocol designed to narrow the range of COHb levels, reproducibility criteria for exercise to eliminate individuals with variant angina, and repeated exercise testing on each test day to reduce the variability in subject response to exercise.

Comparison of the results of this investigation with other studies of the influence of low doses of CO on exercise performance in individuals with coronary artery disease must be approached cautiously because of substantial differences in the studies. Table 27 and Figure 15 compare this study with other studies that evaluated the effect of low doses of CO on time to onset of angina in subjects with coronary artery disease. Table 28 describes the characteristics of the subjects in these studies. Caution should be used in interpreting the percent decrease in time to angina reported by different investigators (Table 27) because the exercise test protocols differed. The subjects in the present study exercised approximately twice as long, presumably because a gradual incremental workload was employed for the exercise stress test. However, insufficient detail is available from most of the other studies to fully substantiate this assumption.

Of the seven studies summarized in Tables 27 and 28, five (*5–9,43*), including our study, reported that low doses of CO decreased the time to onset of angina. This effect was seen at COHb levels of approximately 2 to 3%, measured by optical methods. These optical mea-

surements of COHb are not accurate and should be considered to be within about 1% COHb of the true value. In our study and the Anderson et al. study (*6*), more than one dose of CO was used. Anderson et al. (*6*) found the same decrease in time to angina at both 2.9 and 4.5% COHb, whereas we found a strong dose-response relation. Because of the accuracy of individual COHb values measured by gas chromatography, we were able to use individual COHb levels in assessing the dose-response relationship (Fig. 8).

The Sheps et al. (*17*) and Adams et al. (*18*) studies, which did not show an effect of CO on time to angina, used higher CO doses (4.1 and 5.9% COHb) than the five studies that reported an effect. However, the results of these studies are not incompatible with the studies reporting an effect (Fig. 15). Because of the large standard error, a larger number of subjects would have been needed to detect an effect. In the Adams et al. study (*18*), although no significant changes were observed using conventional statistical procedures, an actuarial analysis, which allowed inclusion of four subjects who developed angina only on the CO day, showed a significant effect of CO on time to angina. In addition, Adams et al. (*18*) reported that left ventricular performance, assessed by radionuclide measurement of the ejection fraction, was reduced during submaximal exercise after exposure to CO compared to air.

The time to onset of ECG ST segment changes, which are thought to be indicative of myocardial ischemia, is a more objective indicator than angina. We found a 5.1% decrease (one-sided $p = 0.01$) at 2.0% COHb and a 12.1% decrease (one-sided $p \leq 0.0001$) at 3.9% COHb in the time to the onset of ischemic ST changes. We also observed an 11 and 17% increase in the magnitude of the maximal ST amplitude at the end of exercise at 2% and 3.9%COHb, respectively, but found no effect on the duration of ST-segment changes. Aronow and Isbell reported that ST-segment depression occurred earlier after CO exposure, compared to normal air, but stated that ST-segment depression was not rigorously quantified (*5*). In addition, an increase from 1.30 to 1.45 mm (not statistically significant) in maximal ST-segment depression was reported after CO exposure. Aronow (*7*) did not report ST data but stated that ST-segment depression appeared to occur earlier with CO exposure, after less exercise, and at a lower heart rate-blood pressure product. Although the ST data were not quantified, Anderson (*6*) stated that "generally, ST-segment depression appeared earlier and was deeper after one or both concentrations of CO, compared with air." Kleinman and Whittenberger (*43*) reported that seven (of 26) subjects "showed small depressions of the ST-segment of their ECGs at the point of angina on both test days, and one subject showed ST-segment depression only on the clean air day." However, the average ST-segment depression reported was only 0.4 mm, which is not considered indicative of myocardial ischemia. Also, the differences were not statistically significant. However, more recently, Kleinman et al. (*19*) reported a statistically significant decrease in the time to 0.1 mV (1 mm) ST-segment depression in eight subjects in the same study. The reason for the discrepancy between these two reports on the same subjects is not clear. Sheps et al. (*17*) did not find an effect of CO exposure on the time to 1-mm ST-segment depression, the maximal ST depression, or the heart rate-blood pressure double product at the time of ST-segment depression. Adams et al. (*18*) reported that time to $\geq$ 1 mm ST-segment depression and maximal ST-segment depression were similar after CO and air exposures.

One important difference among the various studies is in the number of subjects that were evaluated. The number of subjects in our study enabled detection of small effects that would not have been found with a smaller cohort with the variability among subjects seen in our study. Each of the individual centers in this study enrolled approximately as many subjects as did the Sheps study (*17*), and analysis of the data reported by one of them (St. Louis) did not show a reduction in time to angina or time to ST end point at the 2%-COHb target exposure. That center also had the lowest mean COHb levels (by GC) when compared to the other centers (Table 10). Also, the CO effects on angina were not significant at the 5% confidence level at Johns Hopkins on the 4%-COHb-target exposure day. The apparent lack of consistency in results from the three centers is probably due to the relatively small number of subjects and variability in response as well as to differences in COHb levels at the centers (Tables 25 and 26).

The COHb levels reported in most of the previous investigations were measured at the end of exposure, rather than at the time of angina. Decreases in COHb levels may have occurred, depending on the time interval to the beginning of the exercise stress test. The duration and severity of exercise would also affect CO loss caused primarily by exercise-induced hyperventilation. Thus, the ability of the COHb levels measured at the end of exposure to reflect accurately the conditions at the time of exercise-induced ischemia is clearly influenced by these additional factors related to study design. In this study, both postexposure and postexercise COHb levels are reported (Tables 12 and 13), and one can see significant decreases in COHb levels between the two samples.

Another important difference among the studies is whether or not subjects were maintained on anti-anginal medications. Therapeutic approaches and the efficacy of treatment have evolved during the time period spanning these studies. Currently employed therapy may be more effective in preventing ischemia, and studies using these anti-anginal regimens may be important for the development of regulatory standards. In our study, the Kleinman (*19,43*), Sheps (*17*), and Adams (*18*) studies, subjects remained on medications, as indicated in Table 28. In our study, the rationale for this decision was that the subjects should be studied under conditions that approximated the usual level of health care, so that the effects would be representative of those that would occur outside of the experimental laboratory. In the Anderson study (*6*), drug therapy,

such as propanolol and quinidine, was a basis for exclusion from the study, perhaps indicating a milder severity of disease since their subjects did not require anti-anginal medication or could tolerate withdrawal from it. Unfortunately, therapy was not discussed in the Aronow (*7*) or Aronow and Isbell (*5*) reports.

While it is difficult to compare the subject populations by using data from these published reports, the subjects in our study were selected by strictly defined criteria. The purpose of these entry criteria was to assure that all subjects had well-documented coronary artery disease and could provide reproducible experimental data about the ST and angina end points, while still being representative of the general population of individuals with coronary artery disease. In addition to stable exertional angina and a positive exercise treadmill test, all subjects were required to have one or more of three additional indicators of ischemical myocardial disease: angiographic evidence of 70% or more obstruction of at least one coronary artery, documented myocardial infarction, or a positive thallium stress test. In contrast to the other studies, the Anderson study (*6*) included smokers who refrained from smoking for 12 hr prior to exposure, whereas the other studies excluded current smokers. The effects of chronic exposure to CO in cigarette smokers with coronary artery disease are unknown, and inclusion of smokers in that study makes comparison with other studies difficult.

The type of exercise employed to induce angina pectoris differed among these studies. In our study and the Anderson study (*6*), subjects exercised on a treadmill, whereas a bicycle ergometer was used in the other five studies. It is difficult to evaluate the specific exercise protocols because of the lack of detail provided in the other reports. However, inferences can be drawn based upon the performance of the subjects in each study. Under room air conditions, the modified Naughton exercise protocol used in this study resulted in an average duration of exercise prior to the onset of angina of more than 500 sec, compared to 325 sec in the Anderson study (*6*), 312 sec in the Sheps study (*17*), 226 sec in the Aronow and Isbell study (*5*), 323 sec in the Aronow study (*7*), 390 sec in the Kleinman and Whittenberger study (*19,43*), and 288 sec in the Adams study (*18*). Some of these protocols must have employed rapid incremental exercise workloads or studied subjects with severe coronary artery disease who were very sensitive to exercise. Since only the Kleinman (*19,43*) study measured oxygen consumption, it is not possible to determine the actual level of exercise at which angina occurred in all of the studies. Because the exercise protocols and subjects' exercise capabilities varied among the seven studies, comparisons of the percent reduction in time to angina appear to be more useful than comparisons of change in actual time to angina. This is supported by a finding in the present study of similar percent changes among subjects with different exercise capabilities.

The methodology used by other investigators to measure ST segment changes may account for their finding no significant changes in the ECG after CO exposure. In this study the mean decrease in time to ST end point was 30 sec on 2.0% COHb and 69 sec at 3.9% COHb. We measured ST segment changes continuously in 12 ECG leads simultaneously via on-line computer assisted methods. This procedure enabled resolution within 10 sec in the determination of the ST end point. Other investigators collected ECG data every minute; under such conditions a much larger number of subjects would be needed to detect changes. In addition, the incremental nature of the exercise protocol that was used was not chosen according to recommended guidelines for observation of these changes in subjects with stable angina. Redwood et al. (*37*) recommended that increments in progressive exercise tests be no more than 20 watts, which was used in this study. However, it appears that Sheps et al. (*17*), Adams et al. (*18*), and Kleinman et al. (*19,43*) used higher increments as judged by information provided in the reports. These investigators started the subjects at a low level of exercise and then used a large first increment in work to suit operational needs of their study design. For the Sheps et al. study (*17*), the effect of this incremental protocol was to shorten the exercise time to the onset of angina, which has the effect of increasing the variability in the response considerably (*44*). It therefore appears as though some of the inconsistencies in the observations in recent studies are due to study design differences and to the use of measurement techniques of varying sensitivity.

In summary, of the seven studies discussed here, five demonstrated statistically significant effects of low levels of COHb on time to angina by our analyses. For a variety of reasons discussed in this section, including the small numbers of subjects, differences in subject populations, and differences in procedures, methodology, and study design, it is not surprising that two studies did not show an effect on time to angina. None of the other studies provides substantive analysis of ST segment changes, which showed significant effects in this study.

Implications of the Findings

Effects of Carbon Monoxide on Health and Quality of Life: Significance of Results

The clinical significance of these findings must be interpreted with respect to the population studied, the levels of exercise achieved, and the physiological effects of the actual CO exposure. All subjects in this study had evidence of atherosclerotic coronary artery obstruction, which limits coronary blood flow and reduces myocardial function. The increased cardiac output and myocardial oxygen demand required by exercise cannot be met beyond a specific threshold level in these individuals. The results of this study show that CO exposures producing mean COHb levels of 2% pro-

duce detectable changes in the ischemic threshold, as assessed by a reduction in the time to ST end point and the earlier development of angina pectoris. However, the relationship between the magnitude of these changes in exercise function and subsequent alterations in an individual's quality of life is complex and requires careful consideration.

The magnitude of the change in exercise performance produced at 3.9% COHb in this study is similar to that considered clinically significant when evaluating the efficacy of antiischemic therapeutic interventions (*45–49*). While the production of ischemia during exercise is not necessarily related to major cardiac events, such as an increased risk of myocardial infarction or an acceleration of mortality, it is generally agreed that myocardial ischemia is detrimental. Intermittent brief periods of ischemia have a cumulative effect and may cause myocardial necrosis (*50*). In addition, myocardial ischemia can predispose an individual to ventricular arrhythmias (*51*), lower the fibrillation threshold (*52*), and may be related to sudden death (*53*).

The relationship between exercise capacity, as assessed on a treadmill or bicycle ergometer, and the ability to perform work or recreational activities is complicated. Unfortunately, there are no well-controlled, objective studies available to correlate these activities precisely with different levels of treadmill exercise. Thus, one is forced to approximate these relationships. In addition, although exercise level is best quantified by direct assessment of oxygen consumption ($\dot{V}O_2$), it was felt that the changes in experimental methods that would have been required to measure $\dot{V}O_2$ during exercise might have affected the primary cardiac endpoints. Thus, direct measurement of $\dot{V}O_2$ was not performed, but a modified Naughton protocol (*12*) was selected that uses a gradual stepwise increase in workload that correlates with $\dot{V}O_2$. However, because there are individual differences in $\dot{V}O_2$ during treadmill exercise, the precise $\dot{V}O_2$ or METs* used at each level in the modified Naughton protocol can only be estimated. Despite these reservations, it is useful to consider the approximate exercise level at which ischemic changes occurred in this study population. Electrocardiographic evidence of ischemia developed in the average subject at approximately 5 to 6 METs. Thus, ischemia might be expected to occur in this at-risk population when individuals performing at light to moderate levels of activity were exposed to CO that produced COHb levels as low as 2%. These exercise levels might be reproduced by climbing one to two flights of stairs or walking on level, firm ground at a slow to moderate pace (2–3 miles per hour) for a distance of 0.5 to 1 mile. Thus, the subjects recruited for this study are not sedentary, and would be likely to perform different levels of recreational or work activity, but could not engage in heavy work. In those individuals with angina pectoris, exertional activity would be self-limited by the development of chest pain, whereas those with silent ischemia would not experience such a warning signal.

Physicians treating patients with coronary artery disease try to limit physical activity below a known ischemic threshold, usually defined by the results of exercise stress testing. Knowledge of the effect of CO on objective evidence of ischemia during exercise testing (such as time to ST changes) would therefore have an impact on the allowable levels of activity recommended by physicians, and thus on the patient's quality of life, independent of symptoms of angina pectoris. Thus, low levels of COHb produce health effects that can have significant consequences on the functional capacity of an individual performing activities of daily life. These effects occur with light to moderate levels of exercise that are routinely performed by most individuals.

Another important finding of this study was the fact that there was a dose-response relationship between COHb and ischemia without evidence of a measurable threshold effect. Examination of the results that compared the effects of increasing COHb from baseline levels (0.6%) to 2 and 3.9% COHb showed that each increment produced further changes in objective ECG measures of ischemia. This finding implies that even small increments in COHb could adversely affect myocardial function and produce ischemia. Thus, increases in COHb in individuals with high baseline COHb levels, such as cigarette smokers, might produce deleterious health effects. Further study in such exposed population groups is necessary to investigate this possibility.

Demographic Perspective

An important question concerns the applicability of these findings to a general population of individuals with coronary artery disease. While this study was restricted to male nonsmokers with stable angina pectoris and reproducible exercise tests, clearly, the biological effects of CO exposure are unlikely to be limited to this specific population group. Active tobacco smoking was an exclusion criteria for this study because of high COHb levels in smokers. Thus, it does not provide direct information on whether or not smokers with chronically elevated blood COHb levels respond with similar decreases in exercise capacity when incrementally subjected to higher CO levels or, less likely, whether or not they develop tolerance with chronic CO exposure. Although men were chosen to minimize the possibility of false-positive exercise tests, there is no evidence that women with coronary artery disease are less sensitive to CO. In addition, even those individuals with coronary artery disease who do not experience angina during exercise do develop typical ECG evidence of ischemia. Thus, the results of this investigation should be applicable to most individuals who develop ECG changes during exercise treadmill testing.

The fact that all subjects continued to take their usual anti-ischemic medications also makes them rep-

*One MET equals 3.5 mL of O_2 consumed per kilogram body weight per minute and is roughly equivalent to the $\dot{V}O_2$ during resting conditions.

resentative of an ambulatory population receiving optimal medical care for their coronary artery disease. There is no evidence that medications might affect these results, and an analysis showed no differences between subjects who either were or were not receiving beta-adrenergic blockers. It is also possible that some medications were protective and that a less well-managed group might have been more sensitive to the effects of CO exposure. However, it was beyond the scope of this study to address the important questions of possible interactions between antianginal drugs and the responses to CO exposure.

If the subjects in our study are compared with another large, stable-angina, multicenter study population (2982 patients) derived from the Coronary Artery Surgery Study (CASS) registry (*54*), one finds that subjects in this multicenter study are similar in terms of their baseline characteristics: age, gender, angina severity, incidence of prior myocardial infarctions, drug therapy, extent of coronary artery disease, and exercise performance. In addition, it should be noted that these characteristics are also similar to those of groups of individuals with silent ischemia who have ST segment changes without angina during exercise treadmill testing, as well as those who develop angina without ST changes. Thus, the primary results of the study appear to be applicable to a more general population of subjects with coronary artery disease and stable angina pectoris.

There were 6.7 million individuals in the United States with ischemic heart disease, as defined by the U.S. Department of Health and Human Services (*55*) in the National Heart Interview Survey of 1985. Overall, they compose 2.9% of the United States population. This includes individuals known to have silent ischemia as well as those with angina pectoris. The presence of cardiac disease is in part a function of the age distribution of the population in the United States. For example, the frequency of individuals with ischemic heart disease is 61.8/1000 among those ages 45 to 64 years, and 138.5/1000 among those 65 years or older. Because survival in the United States has improved and the population distribution is shifting toward the elderly, the fraction of individuals with age-related diseases, such as ischemic heart disease, is continuing to increase.

Relevance of Exposure Conditions

The CO-exposure protocol in this study was designed to produce specific levels of COHb in subjects at the end of exercise. The lower level, 2.0% COHb, is at the upper end of the range of values expected to occur after 8 hr of exposure to CO at levels meeting the current NAAQS (an average of 9 ppm over 8 hr) (*1*). It is also within the range expected from a 1-hr exposure at 35 ppm (the level of the 1-hr standard) if the subject is exercising moderately (alveolar ventilation of 20 L/min) which thus increases the CO-loading rate. Our exposure protocol was designed to mimic the higher rate of CO uptake over a 1-hr period but in resting subjects. Because our subjects were exposed during rest, a higher level of atmospheric CO (mean 117) ppm) was required to achieve 2% COHb in a period of about 1 hr. To attain 4% COHb, subjects were exposed to mean CO levels of 253 ppm at rest.

The low dose of CO in our study, designed to produce 2% COHb in subjects, was selected for relevance to the NAAQS for CO. In interpreting the impact of our findings on the population, however, it is necessary to know the frequency distribution of COHb levels in the population. Wallace and Ziegenfus (*56*) summarized CO data from 36 outdoor monitors and COHb levels in 1528 nonsmokers in 20 U.S. cities with populations greater than 100,000. The data were collected between 1976 and 1980 as part of the Second National Health and Nutrition Examination Survey (NHANES II). While some cities had several monitors, the subjects were not always geographically localized in relation to specific atmospheric CO monitors. COHb measurements on blood samples from these subjects were made by spectrophotometry and were adjusted so that average values agreed with GC measurements of COHb in a subset of 200 blood samples. The highest 8-hr mean COHb level in the 20 cities studied was 1.6%, found in residents of the District of Columbia, where the two CO monitors had mean levels of 1.8 and 3.5 ppm for the 8-hr period preceding blood sampling for COHb levels. In contrast, the lowest mean group COHb level, 0.55%, was found in Des Moines, Iowa, where the mean CO level was 3.8 ppm. Thus, the average atmospheric CO levels at the monitoring stations in these cities did not correlate well with the measured COHb levels. This finding suggests that indoor or microenvironmental exposure may be more important than the levels measured by conventional atmospheric monitors.

It is also interesting to examine data from individuals with the highest actual COHb levels (*56*). In four of the 20 cities, individuals with COHb levels in the top 5% had mean levels of greater than or equal to 2.0% COHb: District of Columbia (4.6% COHb), San Diego (3.00% COHb), Los Angeles (2.77% COHb), and New York City (Bronx) (2.4% COHb). We do not know whether or not the source of CO in those individuals is ambient air nor can we necessarily equate these COHb levels with our GC values. Nonetheless, the COHb levels in these subjects appear to be in the range that produces adverse effects in individuals with coronary artery disease.

Although many individuals had COHb levels greater than 2% in the 20 cities (*56*), only one of the 36 monitoring stations reported a mean level above the 8-hr average standard of 9 ppm. This occurred at one of the six Manhattan sites, where the CO level was recorded as 13.2 ppm. Two other Manhattan sites had the next highest levels of the 36 sites, at 5.9 and 5.2 ppm. Yet the COHb levels of the Manhattan residents were relatively low (1.04% mean) when compared to the COHb levels of residents of other cities, such as Washington, DC (1.6% COHb). Thus, the CO monitors did not pro-

vide an accurate method to predict COHb levels because they may not have been located near the relevant sources of CO. Furthermore, individual behavior patterns and activity levels may affect COHb levels. For example, three of the most common sources of indoor carbon monoxide are environmental tobacco smoke, gas stoves, and attached garages. It is beyond the ability of atmospheric monitoring to detect these microenvironments that can lead to significant carbon monoxide exposure. Given the limitations of COHb measurement at low concentrations and the inability to relate ambient CO levels in large cities to COHb levels, the true prevalence of biologically significant COHb levels elevations in different urban populations is uncertain. Air-quality standards to limit background CO concentrations (as measured at monitoring stations) are unlikely to prevent localized exposures and are unlikely to prevent all individuals at risk from experiencing elevated COHb.

There are individuals who have high risk occupations relative to exposure to carbon monoxide. Ayres and co-workers (*57*) measured COHb levels in more than 1000 New York City residents. These individuals included people occupationally exposed to high levels of CO, such as policemen, bridge workers, and tunnel workers. Even among nonsmokers, COHb levels were higher than those reported by Wallace and Ziegenfus (*56*). For example, the mean COHb level in nonsmoking policemen working in congested precincts in New York City was 3.4%. Among nonsmoking hospital patients, whose exposure to ambient CO should be relatively low, average COHb levels were 1.56%, compared to the 1.04% reported by Wallace and Ziegenfus (*56*) for Manhattan residents. Some of the differences between the studies could be due to differences in methods of COHb measurement or to differences in the study population.

Smoking is a major contributing factor to elevated COHb levels. In the study by Ayres and co-workers (*57*), policemen from congested areas who did not smoke had COHb levels of 3.14%, whereas policemen from the same areas who did smoke had COHb levels of 8.11%. The NHANES II report describes average COHb levels of 4.53% in smokers and 0.88% in nonsmokers (*58*). The 2.0%- and 3.9%-COHb levels at the end of exercise attained in this study represent levels commonly observed among United States adults who live in urban environments. Smokers may be a particularly sensitive group with respect to CO exposure because of their high COHb levels and increased risk for the development of coronary artery disease. Thus, there are important questions concerning the health effects of CO exposure in smokers and whether or not these individuals develop physiologic adaptations to chronic CO exposure.

In summary, the CO-exposure conditions and the resulting COHb levels in this study appear to be within a realistic range for subsets of the adult, nonsmoking, United States population that are heavily exposed to traffic or other local sources of CO. Unfortunately, such local exposures may be difficult to control with ambient air-quality standards. In addition, since very low levels affect work performance in subjects with coronary artery disease, one must also question whether or not CO exposures would limit the ability to perform work in individuals with vascular disease influences other organ systems other than the heart.

Summary and Conclusions

We report results showing the effects of low doses of CO on myocardial ischemia in 63 subjects with documented coronary artery disease. At the lower dose (2% COHb), the rate of CO uptake in our subjects at rest mimics the projected uptake rate in moderately exercising subjects exposed to CO at the level of the standard (35 ppm).

At mean COHb levels of 2%, subjects had a 5% reduction in the time to onset of ischemic ST segment changes in the ECG, and a 4% reduction in time to onset of angina pectoris, compared to the control day. The 90% confidence intervals were 1.5 and 8.7% for ST and 0.7 and 7.9% for angina for the 2%-COHb analysis. At mean COHb levels of 3.9% subjects showed a 12% decrease in time to ST end point and a 7% decrease in time to angina, compared to the control day. The 90% confidence intervals were 9.0 and 15.3% for ST and 3.1 and 10.9% for angina, for the 3.9%-COHb analysis.

In addition, a significant dose-response relationship was found for the individual differences in the time to ST end point, for the pre- versus postexposure exercise tests, at the three COHb levels ($p \leq 0.0001$). For this range of COHb levels (0.2 to 5.1%), a $3.9 \pm 0.6\%$ decrease in time to ST end point occurred for every 1% increase in COHb. These findings show that ischemia, as measured by both ECG changes and angina, develops at an earlier stage of exercise after low doses of CO exposure than after exposure to air alone.

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Appendix A. Randomization to Exposure

The randomization plan called for stratification by institution, and by whether or not the subjects had a previous myocardial infarction (MI). Within each of these six strata (three institutions times two previous MI statuses), the subjects were balanced in groups of six so that each of the six possible orders of exposure (air, 2%-COHb target, 4%-COHb target) was represented (Table A1). Consequently, each center received two randomization lists, one for MI subjects and one for non-MI subjects. An example of a randomization list is shown in Table A2. Randomized order was used to guard against a learning phenomenon. The results of the orders of exposure are reported in the Methods section of the report. The order of exposure was kept blind to everyone who did not have an initial need to know until after the ECG readings had been made.

The randomization plan was not perfectly obeyed. The Statistical and Data Management Center sent out the randomization lists to the three test centers but, due to a misunderstanding, two of the centers used the sample randomization list contained in the protocol document. Before the error was caught and corrected, Johns Hopkins had registered five subjects with no prior MI and three subjects with MI, and Rancho Los Amigos had registered four patients with no prior MI and seven patients with MI. However, neither the randomization nor the balancing was compromised. The effect of the error was that if someone knew that the wrong randomization list was being used, then the blinding would have been threatened. Since the same people knew which randomization list was being used throughout the study, this error did not add any problems.

Appendix B. Alternative Analyses

Nontrimmed Mean Analyses

The original protocol for the study laid out plans for the primary analyses of the percent effect of CO on time to ST end point and time to angina at two target COHb levels. These plans included the use of trimmed means to guard against outliers. The results of these analyses are presented in the Results section. In this appendix, we present the results of the analyses of percent changes in time to ST end point and time to angina that do not use trimmed means. The results for the 63 subjects who completed all test visits and met the protocol requirements are shown in Tables B1 and B2. These are the subjects on whom the results described in the Results section of the report are based. Results are also presented for 69 subjects, the 63 subjects who are in the main analysis, plus 6 subjects who completed the test visits but did not meet all the protocol requirements in Tables B3 and B4. In all four tables we also present analyses of the change in time between air and CO days, as well as percent change in time to angina and to ST end point.

Table A1. Six possible exposure orderings in three visits.

Ordering	Visit 2	Visit 3	Visit 4
1	Air	2%-COHb target	4%-COHb target
2	Air	4%-COHb target	2%-COHb target
3	2%-COHb target	Air	4%-COHb target
4	2%-COHb target	4%-COHb target	Air
5	4%-COHb target	Air	2%-COHb target
6	4%-COHb target	2%-COHb target	Air

Table A2. Example of randomization list.

Ordering	Visit 2	Visit 3	Visit 4
A	Air	4%-COHb target	2%-COHb target
B	2%-COHb target	Air	4%-COHb target
C	Air	2%-COHb target	4%-COHb target
D	4%-COHb target	Air	2%-COHb target
E	2%-COHb target	4%-COHb target	Air
F	4%-COHb target	2%-COHb target	Air
G	2%-COHb target	4%-COHb target	Air
H	Air	2%-COHb target	4%-COHb target
I	4%-COHb target	Air	2%-COHb target
J	4%-COHb target	2%-COHb target	Air
K	Air	4%-COHb target	2%-COHbtarget
L	2%-COHb target	Air	4%-COHb target
M	4%-COHb target	2%-COHb target	Air
N	2%-COHb target	4%-COHb target	Air
O	2%-COHb target	Air	4%-COHb target
P	Air	4%-COHb target	2%-COHb target
Q	Air	2%-COHb target	4%-COHb target
R	4%-COHb target	Air	2%-COHb target

Table B1. Effect of carbon monoxide on time to ST end point.[a]

Exposure comparison	Change in time to ST comparison between air and CO days, sec			% Decrease in time to ST between air and CO days		
	Mean	p-Value[b]	95% Confidence interval	Mean %	p-Value[b]	95% Confidence interval
Combined data						
Air vs. 2%-COHb target	30.4	0.003	9.20, 51.40	5.1	0.01	0.68, 9.46
Air vs. 4%-COHb target	68.7	≤ 0.0001	47.06, 90.14	12.9	≤ 0.0001	8.42, 17.28
Johns Hopkins University						
Air vs. 2%-COHb target	36.7	0.04	- 3.71, 76.91	6.1	0.04	-0.78, 13.02
Air vs. 4%-COHb target	95.8	≤ 0.0001	53.94, 139.66	16.0	≤ 0.0001	9.50, 22.56
Rancho Los Amigos Medical Center						
Air vs. 2%-COHb target	57.2	0.001	23.52, 90.68	12.9	0.001	5.17, 20.69
Air vs. 4%-COHb target	72.5	0.004	21.70, 123.30	13.5	0.02	
St. Louis University						
Air vs. 2%-COHb target	3.3	0.42	-32.44, 39.04	-2.0	> 0.5	-5.55, 1.45
Air vs. 4%-COHb target	39.9	0.0007	17.37, 62.43	9.3	0.0007	4.07, 14.53

[a] Same 63 subjects as main analysis, nontrimmed means.
[b] One-sided *p*-values.

Table B2. Effect of carbon monoxide on time to angina.[a]

Exposure comparison	Change in time to angina comparison between air and CO days, sec			% Decrease in time to angina between air and CO days		
	Mean	p-Value[b]	95% Confidence interval	Mean %	p-Value[b]	95% Confidence interval
Combined data						
Air vs. 2%-COHb target	25.4	0.018	1.65, 48.95	5.0	0.02	0.10, 9.80
Air vs. 4%-COHb target	32.2	0.006	6.88, 57.52	6.6	0.006	1.52, 11.74
Johns Hopkins University						
Air vs. 2%-COHb target	30.1	0.024	0.26, 59.95	6.2	0.02	0.03, 12.37
Air vs. 4%-COHb target	16.1	0.22	-25.69, 57.69	4.4	0.10	- 2.53, 11.35
Rancho Los Amigos Medical Center						
Air vs. 2%-COHb target	50.3	0.014	5.99, 94.61	11.3	0.01	1.87, 20.77
Air vs. 4%-COHb target	47.7	0.014	5.87, 89.33	9.9	0.02	0.67, 19.11
St. Louis University						
Air vs. 2%-COHb target	0.23	0.50	-50.20, 50.60	- 1.5	> 0.5	-11.30, 8.28
Air vs. 4%-COHb target	35.5	0.08	-15.00, 86.00	6.2	0.13	- 4.81, 17.19

[a] Same 63 subjects as main analysis, nontrimmed means.
[b] One-sided *p*-values.

Comparing the results of Tables B1 and B2 with tables in the results section enables a comparison of the results of the trimmed-mean analyses with the analyses of the percent change in time to ST end point and to angina. With respect to time to ST end point, the differences are small. For example, for the combined data, at 2% COHb, both trimmed and untrimmed means showed a 5.1% decrease; at the 3.9%-COHb the trimmed-mean decrease is 12.1%, and the nontrimmed-mean decrease is 12.9%. Whether or not means are trimmed, 2%-COHb-target exposure did not have an effect at St. Louis, but effects are significant, at $p \leq 0.05$, in all other cases.

With respect to angina, again the picture is similar whether the traditional analyses or the trimmed-mean analyses of percent change are used. For the combined data, the mean change in time to angina at 2% COHb is 5.0%, and the trimmed mean is 4.2%; the mean change in time to angina 3.9% COHb is 6.6%, and the trimmed-mean change is 7.1%. The trimmed-mean analyses give statistically significant effects ($p \leq 0.05$) in all but two instances (4%-COHb target at Johns Hopkins; 2%-COHb target at St. Louis), and the traditional analyses give significant effects except for three instances (the two above and 4%-COHb target at St. Louis).

Comparing Tables B1 and B2 with Tables B3 and B4 shows the difference in results when six subjects

Table B3. Effect of carbon monoxide on time to ST end point in 69 subjects.[a]

Exposure day	Sample size	COHb levels at end of exercise pre- and postexposure			Time to ST end point pre- and postexposure, sec			Change in time to ST end point post- vs. preexposure, sec		Decrease in time between air and CO days, sec				% Decrease between air and CO days			
			Mean %COHb[b]	SEM[c]		Mean	SEM	Mean	SEM	Mean	p-Value[d]	95% Confidence interval		Mean %	p-Value[d]	95% Confidence interval	
Combined data																	
Air	63	Pre	0.63	0.02	Pre	564.4	26.5	16.7	11.5								
		Post	0.61	0.02	Post	581.1	26.6										
2%-COHb target	63	Pre	0.62	0.02	Pre	577.0	27.1	−15.7	13.0	32.3	0.002	11.01,	53.59	5.0	0.01	0.71,	9.37
		Post	2.00	0.05	Post	561.3	25.0										
4%-COHb target	63	Pre	0.64	0.02	Pre	568.2	27.7	−53.8	12.5	70.5	≤ 0.0001	49.01,	91.99	13.0	≤ 0.0001	8.61,	17.33
		Post	3.88	0.08	Post	514.4	25.9										
Johns Hopkins University																	
Air	22	Pre	0.60	0.04	Pre	596.0	48.9	7.1	21.8								
		Post	0.58	0.05	Post	603.1	50.7										
2%-COHb target	22	Pre	0.60	0.03	Pre	601.8	47.7	−29.6	29.3	36.7	0.04	−3.71,	76.91	6.1	0.04	−0.78,	13.02
		Post	2.25	0.06	Post	572.2	47.7										
4%-COHb target	22	Pre	0.65	0.04	Pre	610.1	48.0	−88.7	25.7	95.8	≤ 0.0001	53.94,	137.66	6.0	≤ 0.0001	9.50,	22.56
		Post	3.99	0.12	Post	521.4	46.7										
Rancho Los Amigos Medical Center																	
Air	18	Pre	0.74	0.04	Pre	594.4	45.4	52.2	23.4								
		Post	0.68	0.03	Post	646.7	43.0										
2%-COHb target	18	Pre	0.68	0.05	Pre	627.9	42.9	−13.4	18.2	65.7	0.0007	29.62,	101.78	13.4	0.0007	6.08,	20.82
		Post	2.08	0.09	Post	614.4	40.6										
4%-COHb target	18	Pre	0.73	0.05	Pre	605.7	54.5	−26.3	25.8	78.5	0.002	29.40,	127.60	13.9	0.014	1.70,	26.16
		Post	4.12	0.13	Post	579.4	47.7										
St. Louis University																	
Air	23	Pre	0.58	0.04	Pre	510.7	42.3	−2.0	13.8								
		Post	0.58	0.04	Post	508.7	40.0										
2%-COHb target	23	Pre	0.59	0.04	Pre	513.4	46.5	−4.1	17.6	2.1	0.45	−32.00,	36.20	−2.6	0.25	−10.20,	5.08
		Post	1.71	0.07	Post	509.3	39.3										
4%-COHb target	23	Pre	0.57	0.04	Pre	498.8	41.1	−41.9	11.0	39.9	0.0007	17.37,	62.43	9.3	0.0007	4.07,	14.53
		Post	3.59	0.13	Post	456.9	38.6										

[a] Including subjects excluded from main analysis.
[b] CO measured by GC.
[c] SEM, standard error of the mean.
[d] One-sided p-values, as described under "Methods."

Table B4. Effect of carbon monoxide on time to angina in 69 subjects.[a]

Exposure day	Sample size	COHb levels at end of exercise pre- and postexposure			Time to angina pre- and postexposure, sec			Change in time to angina post- vs. preexposure, sec		Decrease in time between air and CO days, sec			% Decrease between air and CO days		
			Mean %COHb[b]	SEM[c]		Mean	SEM	Mean	SEM	Mean	p-Value[d]	95% Confidence interval	Mean %	p-Value[d]	95% Confidence interval
Combined data															
Air	69	Pre	0.64	0.02	Pre	525.1	25.7	−14.7	10.6						
		Post	0.61	0.02	Post	510.4	24.4								
2%-COHb target	69	Pre	0.62	0.02	Pre	531.2	24.9	−42.6	10.2	27.9	0.005	6.21, 49.59	5.3	0.009	0.93, 9.75
		Post	1.99	0.05	Post	488.6	21.3								
4%-COHb target	68	Pre	0.64	0.02	Pre	516.3	26.0	−51.5	10.4	34.2	0.003	10.02, 58.38	7.0	0.0035	1.99, 12.09
		Post	3.87	0.08	Post	472.1	24.2								
Johns Hopkins University															
Air	22	Pre	0.60	0.04	Pre	562.2	57.5	−46.1	16.9						
		Post	0.58	0.05	Post	516.1	51.2								
2%-COHb target	22	Pre	0.60	0.03	Pre	547.7	53.9	−76.2	19.6	30.1	0.024	0.26, 59.95	6.2	0.025	0.03, 12.37
		Post	2.25	0.06	Post	471.5	46.1								
4%-COHb target	22	Pre	0.65	0.04	Pre	557.1	51.7	−62.2	18.9	16.1	0.22	−25.69, 57.69	4.4	0.1	−2.53, 11.35
		Post	3.99	0.12	Post	498.9	49.4								
Rancho Los Amigos Medical Center															
Air	23	Pre	0.74	0.03	Pre	565.0	39.5		17.0	20.4					
		Post	0.69	0.03	Post	582.0	39.6								
2%-COHb target	23	Pre	0.67	0.05	Pre	580.1	37.8	−34.7	16.4	51.6	0.004	15.49, 87.92	10.8	0.0038	3.20, 18.30
		Post	2.02	0.09	Post	545.5	34.0								
4%-COHb target	22	Pre	0.70	0.04	Pre	538.3	46.1	−44.2	18.9	54.7	0.004	16.65, 92.55	11.7	0.005	2.81, 20.54
		Post	4.05	0.14	Post	514.6	46.4								
St. Louis University															
Air	24	Pre	0.58	0.04	Pre	452.8	32.7	−16.3	15.7						
		Post	0.57	0.04	Post	436.5	31.2								
2%-COHb target	24	Pre	0.58	0.04	Pre	469.3	35.3	−19.4	15.6	3.2	0.45	−43.11, 49.51	−0.6	0.44	−9.63, 8.37
		Post	1.7	0.06	Post	449.8	28.7								
4%-COHb target	24	Pre	0.59	0.04	Pre	458.8	36.7	−48.2	16.8	32.0	0.09	−16.93, 80.93	5.2	0.16	−5.51, 15.91
		Post	3.59	0.13	Post	410.5	26.7								

[a] Including subjects dropped from main analysis.
[b] CO measured by GC.
[c] SEM, standard error of the mean.
[d] One-sided p-values, as described under "Methods."

who did not meet the protocol requirements are included in the analyses. With respect to the analyses of percent change in time to ST end point, most of the subjects who did not meet protocol requirements provided no ST data. Thus, it is not surprising that the results are similar; the ST analyses of 69 subjects compared to 63 subjects actually add only two subjects at the 2%-COHb-target exposure and one at the 4%-COHb target. However, in the angina analyses, eight are added at the 2%-COHb-target level and six at the 4%-COHb-target level of exposure. Nonetheless, the results are similar, and the differences are statistically significant in the same instances.

All four tables also present the results of analyses of change in time to angina and to ST end points between air- and CO-exposure days. These results are similar to the analyses of percent change, as is seen by comparing the *p*-values under the last two headings in Tables B1, B2, B3, and B4. In the ST and angina analyses of 63 subjects (Tables B1 and B2), there was a statistically significant effect in all the same instances, whether difference in time or percent difference in time was used to compare results on air- and CO-exposure days. In the analysis of 69 subjects (Tables B3 and B4), there was, again, a statistically significant effect in all the same instances, for both the ST and angina analyses, when comparing time difference to percent time difference beween days.

Randomized Analysis Allowing for Blocking

The order in which the exposures were administered to each subject was tested and found not to be significant. As a further examination of the effect of the order of exposure, randomization tests were performed to account for the possible induced correlation.

As explained in Appendix A, subjects were stratified by whether or not they had had a prior myocardial infarction. Then, within strata, the order of exposure was assigned, at random, in balanced blocks of the six possible orders. The randomization *p*-values reported in Table B5 are a result of pairing subjects within blocks. The pairing within each block was determined by when the "other" exposure was administered. So, for example, for the 2%-COHb-target analysis, the two within a block who had the 4%-COHb-target exposure on the first experimental visit were paired, the two who had the 4%-COHb-target exposure on the second experimental visit were paired, and finally, the two who had the 4%-COHb-target exposure on the third experimental visit were paired. If the pairing could not be done because of a block not being filled, the observation was not paired. This pairing based on when the "other" exposure was administered yields an unbiased test, even if the order—or the blocking—is important.

In the randomization, the two responses "exposure day minus air day" for each pair of subjects were both multiplied by +1, or by −1, with equal probability.

These analyses were only performed for the centers where the samples were relatively small, and where the effect of blocking should be felt the most. An examination of Table B5, in comparison with Tables 19, 20, B1, and B2, shows that except for the time-to-angina *p*-value for the air versus 2%-COHb-target exposure, the three analyses yield very comparable results. The smallest and largest *p*-values in Table B5 are proportionally different by the same amounts from the corresponding *p*-values in Tables 19, 20, B1 and B2.

Table B5. Effect of carbon monoxide on time to ST end point and time to angina: block-randomized *p*-values.

Analysis	Time-to-ST *p*-value	Time-to-angina *p*-value
Johns Hopkins University		
Air vs. 2%-COHb target	0.042	0.08
Air vs. 4%-COHb target	0.0005	0.14
Rancho Los Amigos Medical Center		
Air vs. 2%-COHb target	0.001[a]	0.004
Air vs. 4%-COHb target	0.01	0.02
St. Louis University		
Air vs. 2%-COHb target	> 0.5	>0.5
Air vs. 4%-COHb target	0.0049	0.045

[a] Most extreme attainable in this analysis.

Appendix C. Effectiveness of Exposure Protocol

Minimizing the range of end-of-exposure COHb levels in this study was desirable because the primary

Table C1. Comparison of actual COHb results with projected results if a fixed-concentration/fixed-time protocol had been used.

	2%-COHb-target day		4%-COHb-target day	
	Actual %COHb[a]	Calculated %COHb[b]	Actual %COHb[a]	Calculated %COHb[b]
Mean	3.1	3.2	5.4	5.6
Median	3.1	3.1	5.6	5.8
Range	2.7–3.6	2.1–4.4	4.4–6.0	4.4–7.7
SD	0.26	0.59	0.41	0.79
SEM[c]	0.05	0.12	0.08	0.16

[a] Actual experimental data (individual CO levels/variable time).
[b] Calculated data using uptake rates determined at visit 1 and assuming a single CO-exposure level and constant time.
[c] SEM, standard error of the mean.

analysis compared cardiovascular changes at 0.7% COHb to changes at 2.0% and 4.0% COHb. The more these CO doses could be cleanly separated, the better. The extent to which the protocol maneuvers, of varying exposure concentration and time, were effective in accomplishing this reduction in variability can only be estimated. We have taken two approaches to estimating the scatter that would have been obtained if these subjects had been exposed to fixed levels of CO for a fixed time.

The first approach was to use the data from visit 1, when all subjects were exposed to 150 ppm for 1 hr. The range of COHb values obtained after 60 min of exposure demonstrates considerable variability among individuals in CO uptake. Analysis of data from visit 1 from the St.Louis center shows that, for 24 subjects studied, the end-of-exposure COHb values ranged from 3.8 to 5.8%, with a mean of 4.1% (SD = 0.49). This range of 2.0% among subjects at the end of a 60-min exposure equals the difference between the desired levels after exposure to CO used in this study. Therefore, it suggests that the use of a fixed-exposure/fixed-time protocol would have been unacceptable.

A second approach was the use of the individual uptake rates actually measured on visit 1, combined with the actual starting COHb levels, to project a theoretical end-of-exposure COHb. The predicted levels of COHb for the St. Louis subjects were calculated for fixed atmospheric levels of CO for 60 min of exposure. The calculated end-of-exposure levels were obtained by using the average chamber levels of 102 ppm and 237 ppm CO that were used in the study. The uptake-rate constants determined during the first visit were used to predict the 60-min values. The values of %COHb determined prior to exposure on each day were used as the start values. This process predicts the effect of exposing these subjects to a constant level of CO for 60 min given the variability in their baseline levels of %COHb. The calculated results are compared with the actual results in Table C1. The range, standard deviation, and standard error are all reduced about 2-fold at each target level in the actual, compared to the calculated, results.

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